Basel 2014
SETAC Europe

SCIENCE ACROSS BRIDGES, BORDERS AND BOUNDARIES

ABSTRACT BOOK

SETAC Europe
24th Annual Meeting
Basel, Switzerland,
11-15 May 2014
basel.setac.org

ABSTRACT BOOK
SETAC Europe 24th Annual Meeting

TABLE OF CONTENTS

This book composes the abstracts of the presentations for the platform and poster sessions of the 24th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the Congress Centre Basel, MCH Messe Schweiz (Basel) AG, in Basel, Switzerland from 11-15 May 2014.
The abstracts are reproduced as accepted by the scientific committee of the meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts and poster corners abstracts are included in the list of poster abstracts. The presenting author of each abstract is underlined.

Basel 2014
SETAC Europe

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ISSN 2309-8031

SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists—biologists, chemists, toxicologists—as well as managers and engineers others interested in environmental issues.. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void. Based on the dynamic growth in the Society's membership, meeting attendance and publications, the forum was clearly needed. SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1990, and in Brussels, Belgium, established in 2003.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers, World Council, Geographic Unit Boards of Directors and Councils, and Committee members and governance of activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality through Science®, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. SETAC's growth has been marked the establishment of geographic units around the world: SETAC Europe in 1989, SETAC Asia/Pacific in 1997, SETAC Latin America in 1999 and SETAC Africa in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters of the geographic units are being considered for a number of countries.

Publications

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1980 to a monthly publication of nearly 3,000 pages annually.

Integrated Environmental Assessment and Management, launched in 2005 to bridge the gap between scientific research and it application in environmental decision-making, regulation and management, has become a well-respected quarterly publication of 700 pages annually.

SETAC Books total more than 100, encompassing workshop results and other scientific studies.

Keynote Abstracts

Persistent Organic Pollutants in Switzerland and globally: problems solved?
Martin Scheringer

Persistent Organic Pollutants (POPs) have been a topic of environmental chemistry and toxicology since the 1960s. This year, we celebrate the 10th anniversary of the Stockholm Convention on POPs. Why should POPs, after so many years, still be a research priority within SETAC? First, POPs still cause serious environmental impacts and will continue to do so. Emission sources of POPs are active in many countries and need to be identified and eliminated. The global distribution and long-term fate of POPs poses many open questions; it is currently not known if and under what conditions the environmental reservoirs of POPs may be relevant as secondary emission sources. Moreover, the total number of POPs that need global action is unknown; there might be (many) more than the 23 POPs currently covered by the Stockholm Convention - but which ones? Finally, how can we determine whether the Stockholm Convention is effective? Does our POPs monitoring have sufficient spatial and temporal coverage?

About Martin Scheringer
Martin Scheringer works with the Swiss Federal Institute of Technology (ETH) in Zürich, Switzerland. His field of research is hazard and risk assessment for chemicals with a focus on environmental exposure assessment. Martin Scheringer has developed a suite of multi-media mass-balance models for analyzing the environmental fate of chemicals on various scales from local to global. Key areas of his work are the persistence and long-range transport of chemicals in the environment and the assessment of chemical property data for hazard assessment under REACH. In addition to his research, Martin Scheringer has worked extensively on the science-policy interface. He has conducted several projects with UNEP and is a co-author of a chapter on Chemicals and Waste in UNEP's 5th Global Environment Outlook and a chapter on Minimizing Chemical Risks in UNEP's Year Book 2013. Martin Scheringer is a founding member and currently the chair of the International Panel on Chemical Pollution, IPCP.

Epigenetics in Environmental Toxicology and Chemistry
Juliette Legler

How is possible that exposure to a chemical during development affects a tissue in a way that its function is changed long after the exposure has stopped? How do chemicals program an organism during development, making it more susceptible to diseases later in life? If we are to understand this we must dig down into the very basics of life and how genes work. The central dogma that genes flow in a linear fashion from DNA sequence to messenger RNA to protein is clearly too simplistic. One area of molecular biology where new discoveries are made at an astonishing rate is the field of epigenetics. Epigenetics describes the array of chemical markers and switches that lie along DNA providing instructions to genes for what to do, and where and when to do it. Newfound insights in this field will help us understand how chemicals may alter basic processes in development at levels that may not produce overt toxicity. It is an exciting time in molecular and evolutionary biology, and role of epigenetics in toxicity is the challenge than now lies with us as environmental toxicologists and chemists.

About Juliette Legler
Juliette Legler is a Professor of Toxicology and Environmental Health at the Institute for Environmental Studies of the VU University Amsterdam. With a background in environmental toxicology and molecular biology, she has developed test methods to identify the effects of endocrine disrupting chemicals. Her research with zebrafish and in vitro models has expanded to understanding the effects and underlying mechanisms of chemical exposure during development in both fish and in humans. As coordinator of the EU project OBELIX and recipient of a Netherlands Organization for Scientific Research VIDI grant, she is examining the role of chemical exposure in the developmental origins of health and disease. Legler also coordinates the VU's Master program in Environmental Chemistry and Toxicology and has served on various advisory and review boards, including the Dutch Health Council and OECD.

Sufficiency: Enough but Not Too Much
Thomas Princen

Growth. Efficiency. Consumer sovereignty. These principles served the 20th century well. Resources were abundant, waste sinks vast, demand unyielding. But this is the 21st century. We need to figure how to live within the regenerative capacities of biophysical and social systems. We need to put a brake on endless expansion and consumption. For that, new principles are needed to guide human organization onto a sustainable path. Among them are sufficiency: living well now and into the future by living on less than the most possible now. Thomas Princen will develop the notion of sufficiency grounding it at three levels of behavior—the individual, the organizational, and the societal. He will argue that sufficiency is but one element in a broader "politics of urgent transition.

About Thomas Princen
Thomas Princen explores issues of social and ecological sustainability at the School of Natural Resources and Environment at the University of Michigan. He works on principles for sustainability (e.g., sufficiency), overconsumption, the language and ethics of resource use, and the transition out of fossil fuels.
Princen is the author of Treading Softly: Paths to Ecological Order (2010/2013), author of The Logic of Sufficiency (2005), and lead editor of Confronting Consumption (2002), all three published by MIT Press. The last two were awarded the International Studies Association's Harold and Margaret Sprout Award for the "best book in the study of international environmental problems."
He is co-editor of The Localization Reader: Adapting to the Coming Downshift (MIT Press, 2012), co-author of Environmental NGOs in World Politics: Linking the Local and the Global (Routledge, 1994) and author of Intermediaries in International Conflict (Princeton University Press, 1992/1995).
*Princen is currently working on three book-length projects: *Ending the Fossil Fuel: Keep Them in the Ground (contract, MIT Press), *Distant Horizons: An Ethic of the Long Term *The Politics of Urgent Transition*
He was recently awarded a writing fellowship at the Rachel Carson Center in Munich. Before that he was named an Aldo Leopold Leadership Fellow, sponsored by the Packard Foundation, and before that was a Pew Faculty Fellow for International Affairs.
Princen received his Ph.D. in Political Economy and Government from Harvard University in 1988 and a Bachelor of Arts in biology from Pomona College in 1975. He was a MacArthur Foundation Post-Doctoral Visiting Research Fellow in International Peace and Security at Princeton University from 1988 to 1989. He serves as Faculty Associate, Program in the Environment; Faculty Affiliate, Erb Institute for Global Sustainable Enterprise; Co-Director, Workshop on Urgent Transitions; and Associate Professor of Natural Resource and Environmental Policy; all at the University of Michigan.

Platform Abstracts

Ecotoxicology in tropical and polar regions (I)

1

The Laguna Madre de Dios, a Costa Rican tropical coastal lagoon ecosystem at risk

L.E. Castillo, Universidad Nacional Costa Rica / IRET; C. Ruepert, Universidad Nacional / IRET; F. Ramirez, Universidad Nacional / Central American Institute for the study of toxic substances IRET; G. Moraga, D. Ballestero, C. Brenes, S. Vargas, R. Benavides, Universidad Nacional; F. Mena Torres, Universidad Nacional / IRET; M. Arias, Universidad Nacional; J. Gunnarsson, Stockholm University / Department of Ecology Envionment and Plant Sciences DEEP

The Laguna Madre de Dios is a coastal lagoon located in the Caribbean watershed of Costa Rica. The lagoon has great ecological value but is submitted to anthropogenic stress mainly because of an intensive use of fertilizers and pesticides in the banana, rice and pineapple plantations located upstream. The study presented here is part of a broader study on pesticide risk assessment that includes pesticide runoff, a toxicity assessment of pesticides used in plantations in the area of the RMD Basin; an *in situ* toxicity and ecological assessment of pesticide run-off and the application of the PERPEST and TRIAD methodology. Land and pesticide use studies were carried out using GIS. Pesticide use information was collected through interviews. An hydrological and bathymetric study were carried out at the lagoon in dry and rainy weather.. Sampling sites for pesticide residues and nutrient analysis were selected to cover canals and creeks that flow into the River Madre de Dios (RMD) collecting runoff from the agricultural areas. The main pesticides found in the area include insecticides/nematicides, herbicides and fungicides (amethrine, chlorpyrifos, chlorothalonil, diazinon, diuron, epoxiconazole, ethoprophos, hexazinone and pirimetanil) in concentrations frequently higher than the Water Quality Criteria for the protection of aquatic organisms. Twenty three marine, estuarine and freshwater fish species were identified during one sampling occasion in the lagoon in 2012. Reports of 18 fish kills have been documented in the area for the years 2007-2009, several insecticides and difenoconazole were present in the samples analyzed, reported in the area per year between 2007-2012. The analysis of several fish kills reported during 2013 show the presence of 5-6 pesticides present in those samples including organophosphates such as terbufos and fenamifos as well as diazinon, which are highly toxic compounds. The frequent ocurrence of pesticides in the lagoon in concentrations over the criteria for the protection of aquatic organisms points out the risk of ecological damage to this ecosystem as is confirmed by the fish mortalities ocurrig in the area.

2

TIER I Toxicity assessment of pesticides used in banana and pineapple plantations detected on the Río Madre de Dios watershed, Costa Rica

M. Arias, F. Mena-Torres, R. Ugalde, Universidad Nacional; R. Ramo, Stockholm University; L. Grandas, Universidad Nacional; C. Ruepert, Universidad Nacional / IRET; L. Castillo, Universidad Nacional; P. van den Brink, AlterraWageningen UR / Aquatic Ecology and Water Quality Management Group b Alterra; J. Gunnarsson, Stockholm University / Department of Ecology Envionment and Plant Sciences DEEP

Pesticide runoff in developing countries of tropical regions pose high risks to aquatic ecosystems, due to poor agricultural practices and the use of highly toxic compounds. Costa Rica is among the countries that import higher amounts of agricultural pesticides and environmental toxicity to aquatic ecosystems have long been a concern for communities near intensive areas of banana and pinneaple production. The Río Madre de Dios (RMD) watershed in the Caribbean lowlands is one of the aquatic ecosystems in the Caribbean area where extensive banana and pinneaple plantations exist. This ecosystem has been the focus of attention for several years now as a consequence of fish kills reported with a certain frequency and potentially associated with pesticide runoff. The purpose of this work was to perform a first tier assessment of the toxicity risks of the maximum pesticide concentrations (MEC) found at RMD, by using acute toxicity hazard concentrations for 5% of the species (HC5) and native species toxicity values. Risk Quotients (MEC/HC5≥1.00) were found for the insecticides carbofuran, chlorpyrifos, diazinon, ethoprophos, carbaryl and terbufos and for the herbicide diuron. Toxicity values (LC50-96hrs) for *Parachromis dovii* (fish) were 242 (179-328 95%CI) μg/L for ethoprophos, 202 (140-242 95%CI) μg/L for carbofuran and 117 (87-158 95%CI) μg/L for clorpyriphos. In the case of crustaceans, for *Daphnia ambigu a*EC50-48hrs values of 1.1 μg/L (± 0.8, SD), 0.3μg/L (±0.2, SD) and 0.14 (± 0.001, SD) were found for carbofuran, diazinon and clorpyriphos respectively. Meanwhile, for *Macrobrachium* sp. values of 20 μg/L (12 – 3395%CI), 23.8μg/L (13.4 - 35,895%CI) and 24μg/L (15 – 3895%CI) were found for ethoprophos, carbofuran and diazinon. Finally, the acute toxicity (EC-7d) of diuron to *Lemna* sp. was found to be 31μg/L (±12, SD) and 16μg/L(±3.2, SD) based on reproduction and dry weight measurements.According to acute environmental quality criteria derived from ecotoxicological databases and from the sensitivity of native species measured in the lab, the pesticide concentrations in the RMD basin can have toxic effects on aquatic biota. An ecotoxicological evaluation is the next step to corroborate if the toxicity potential seen can be measured in the field, and if other impairments on community structure and/or function can be detected. **\n**

3

Integrated ecological risk assessment of pesticide run-off using the TRIAD weight-of evidence approach: a case study in the Rio Madre de Dios River, Costa Rica

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The “TRIAD” an ecological risk assessment (ERA) tool based on the combined weight of evidence (WoE) from several lines of evidence (LoE) was used in order to assess the ecological risks from pesticide contamination in the Rio Madre de Dios (RMD), a watershed that drains water from banana and pineapple plantations in the Caribbean lowlands of Costa Rica. The TRIAD is a tiered ERA method, where contamination risks are assessed based on the integration of data from three independent LoE from: 1) chemistry, 2) toxicology, 3) ecology. Within a research project called “TROPICA” (Tiered Risk Assessment of Pesticide Use in Costa Rican Agriculture) chemical, toxicological, and ecological measurements were carried out ca 2-3 times a year in the Rio Madre de Dios, a river system that receives drainage water from banana and pineapple plantations. In two companion studies of the TROPICA project, TIER 1 and TIER 2 ERA analyses were done using toxicity testing in the laboratory and *in situ* respectively, in order to assess the risks from pesticide contamination in the RMD watershed. In this study we gathered all chemical, toxicological and ecological data from these two studies over a sampling period of 3-4 years, scaled all the data from 0-1 values, where 0 corresponds to the minimum and 1 to the maximum risk values, using various scaling techniques. Then the scaled data from the three independent LoE were integrated using geometric means and contamination risk values and their standard deviations were calculated for each sampling season and for the total project duration. Our results show that the RMD river is at high chemical risks from both pesticides (0.7) and nutrients (PO_4) (0.6 - 0.7), that the toxicity risk to aquatic organisms is low (0.1 - 0.2) (based on biomarker and toxicity tests), and that the ecological risks to benthic invertebrates is relatively high (0.5 – 0.7), but not due to pesticide concentrations but rather due o low oxygen levels and eutrophication. Overall the integrated contamination risk to native aquatic organisms in the RMD was moderate (0.4) to high (0.6) indicating that mitigation measures are imperative and urgently needed in order to protect the native wildlife from pesticide and nutrient contamination.

4

Integrated Ecological Risk Assessment (ERA) of aquaculture and anthropogenic pollution in the Phu Long commune, Hai Phong city, Vietnam

P. Bruce, Stockholm University / Department of Ecology Envionment and Plant Sciences DEEP; V. Le, Stockholm University / Ecology Environment and Plant Sciences; H. Than Thi, MCD Vietnam; L. Nguyen Thi Tuyet, HACEM; J. Gunnarsson, Stockholm University / Department of Ecology Envionment and Plant Sciences DEEP

With increased globalization, climate change, high development rates and a growing population several Southeast Asian countries, such as Vietnam, face increasing environmental threats with a following need for preventive and remediation strategies. For the first time in Vietnam, we have applied an adapted version of the TRIAD, an ecological risk assessment (ERA) tool based on the combined weight of evidence (WoE) from several lines of evidence (LoE) and tiered approach ensuring an effective use of resources in order to assess the risks from anthropogenic pollution on the marine environment off Cat Ba island Biosphere Reserve. In a TRIAD data from different LoE were collected and then scaled from 0-1. Integrated risk values were then determined to provide evidence based management options. In the present study the TRIAD method was modified in order to also include socio-economical data, gathered from interview studies Physico-chemical measurements (nutrients, contaminants, O2, suspended solids) together with ecological/biological measurements (community structure of phyto-, zooplankton and benthic fauna) were gathered from three different coastal sites with various aquaculture pressures, and integrated with the TRIAD to interview data from the local population.The results show medium to high risk for several physico-chemical properties and concentrations such as heavy metals, nitrogen and coliforms. The ecological LoE shows low to medium risks for zoobenthic- and plankton communities and abundance and high risk for economic activities such as aquaculture and fishery. When the LoE are integrated, the environmental risk seems to be intermediate or low with acceptable levels of uncertainty. The quality of the assessment relies on good references, such as comparable sites with desirable

environmental status, earlier studies for background levels and relevant environmental quality criteria (EQC). Since a majority of the environmental quality criteria used in Vietnam derive from studies conducted in temperate regions it is questionable if they are suitable criteria in the tropical environment of Southeast Asia and this study shows that local site-specific environmental criteria as well as more background data from monitoring programs are needed in order to assess the ecological risks from growing human activities on coastal marine resources in Vietnam.

5

Ecological risks of antibiotics applied in freshwater cage aquaculture – a case study in tropical Thailand

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Intensive aquaculture relies on the use of chemicals for the prevention and treatment of disease outbreaks, and constitutes one of the most important pathways for antibiotics into the environment. In this study we investigated the use, environmental fate and ecological risks posed by the use of antibiotics in tilapia cage farming in tropical Thailand. The use of antibiotics was investigated through interviews to 29 farmers, and concentrations of the most commonly used antibiotics, enrofloxacin (ENR) and oxytetracycline (OTC), were monitored in water and sediment samples collected in the Tha Chin and Mun Rivers. The toxicity of ENR and OTC was assessed by performing laboratory experiments with tropical freshwater invertebrates. Furthermore, the toxicity of ENR was assessed on five structural (macroinvertebrate, zooplankton, phytoplankton, periphyton and bacterial communities) and two functional (organic matter and nitrogen cycling) ecosystem endpoints by performing a microcosm experiment simulating the antibiotic exposure patterns found in the environment. All the interviewed farmers reported to routinely use antibiotics. Maximum antibiotic concentrations in the river water were 1.6 and 49 μg/L for ENR and OTC, respectively. Antibiotic residues were found in all the collected sediment samples, with concentrations between 1.4-2,339 μg/kg d.w. for ENR, and 4.5-4,062 μg/kg d.w. for OTC. The results of the laboratory experiments showed that tropical invertebrates are only moderately sensitive to water ENR and OTC concentrations. The results of the microcosm experiment did not show consistent treatment-related effects on the structure of the primary producer and invertebrate communities, however, demonstrated a reduction on the abundance of ammonia-oxidizing bacteria and archaea at measured environmental concentrations. Nitrification was only affected at concentrations above 100 μg/L, indicating that the aquatic exposure to ENR might have limited or no significant consequences for ecosystem functioning. We conclude that the ecological risks posed by antibiotic residues to primary producer and pelagic invertebrate communities seem to be minimal, however, more research is needed to assess the effects on benthic communities chronically exposed to antibiotic mixtures. The results of this study can be extrapolated to other tropical regions and contribute to further our understanding on the ecological risk assessment of antibiotics for tropical aquatic ecosystems.

6

Effects of Chlorpyrifos ethyl on cholinesterase activity in Climbing perch (Anabas testudineus , Bloch, 1792), from rice-fields in the Mekong Delta, Vietnam

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Abstract Climbing perch (*Anabas testudineus,* Bloch, 1792), is commonly harvested in rice-fields in the Mekong delta. Despite its importance in providing food and income to local households there is little information about how this species is affected by the high use of pesticides in rice farming in the Mekong Delta. Organophosphate insecticides, such as chlorpyrifos, are commonly used and are highly toxic to aquatic organisms. This study show that brain AChE activity of climbing perch fingerling placed in cages within rice fields was significantly inhibited by single application of chlorpyrifos The water concentration of chlorpyrifos dropped below the detection limit within 3 days, but the inhibition of brain AChE activity remained for several more days. In addition, the chlorpyrifos treatments had a significant impact on survival and growth rates of climbing perch fingerlings, which was proportional to the exposure level. The results indicate that the high use of pesticides in the Mekong Delta could have negative impact on aquatic organism, fish yields and the aquaculture industry in the Mekong Delta.
Keywords: *Climbing perch; Chlorpyrifos; acetylcholinesterase, Mekong Delta*

Marine and coastal ecotoxicology and risk assessment (I)

7

Combined effects of two emerging environmental stressors (PFOS and CO2) on estrogenic and biotransformation responses of Atlantic cod (G. morhua)

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Until now, little effort has been put into investigating interactive effects between POPs and elevated CO2 levels (hypercapnia) in the aquatic environment. In the present study, juvenile Atlantic cod were exposed to PFOS (0, 100 and 200 µg/L) for 1 hour/day in 5 days, followed by changes in elevated water CO2 saturation (0, 0.3 and 0.9%) for 3, 6 and 9 days. Endocrine disrupting potential of PFOS and elevated CO2 levels, both singly and in combination, were examined, in addition to steroid and xenobiotic metabolism (CYP1A, CYP3A) and HIF-1a. Elevated CO2 produced increased levels of E2, T, 11-KT with concomitant increases in mRNA expression of estrogen responsive genes. PFOS produced a weak time- and concentration-dependent estrogenic effect, but no effect on steroid hormone levels. Exposure to combined CO2 and PFOS produced gene expression patterns that are different from the effects observed for CO2 or PFOS alone, indicating interactive effects. Principal component analysis (PCA) did not produce significant components at day 3. The PCA for day 6 and 9 produced the best model fit, where particularly, the individual scores from the different CO2 scenario exposure groups tended to group separately. The resulting model contained three principal components (PCs) explaining a total of 56.4% of the total variation. When pooling the various exposure groups within each CO2 exposure scenarios, scores of three resulting CO2 groups were significantly separated along the first principal component (PC1; Q^2=0.064) with increasing CO2 saturation (p[normal vs moderately increased CO2]=0.029, p[moderately vs highly increased CO2]=0.020), p[normal vs highly increased CO2]=0.000). PC1 mainly explained the individual variation within transcript levels of the estrogenic response genes, followed by levels of sex steroid hormones. These observations suggest that hypercapnia and emerging POPs such as PFOS in combination could modulate the estrogen signaling in juvenile Atlantic cod, with potential consequences for sexual development and reproduction. These findings suggest a potential for adverse effects of increased anthropogenic CO2 emissions on fish reproduction. This also raises the question whether such interactive effects might be observed in other aquatic species and with other endocrine disrupters and POPs as well. Such findings could have implications for the accuracy of current risk assessments of emerging POPs, under changing climatic conditions.

8

Parental exposure to environmental concentrations of diuron leads to aneuploidy in embryos of the Pacific oyster as evidenced by fluorescent in situ hybridization

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Over 80% of marine pollution comes from the mainland and originates from industrial, agricultural and urban activities. France is the leading user of agrochemicals in Europe. As a result of this high consumption of phytosanitary products, pesticides can contaminate coastal waters through various processes, including run-offs, leaching and spray drift. Genotoxicity is common to several families of major environmental pollutants, among them pesticides, which represent therefore a potential major environmental hazard for marine organisms. To investigate these points, the GIMEPEC (Genotoxicity, IMmunotoxicity and rEprotoxicity of Pesticides in *Crassostrea gigas*) project has been set up to study in the Pacific oyster the genotoxic effects of an herbicide, diuron, on the genome of genitors and, the possible transmission to the offspring of damaged DNA and its consequences on oyster fitness. Following exposure of genitors in active gametogenesis to environmental concentrations of diuron to two 7 day-exposure pulses, the genotoxicity of diuron was observed in both genitors, their gametes and the offspring using two complementary approaches, comet assay and flow cytometry. In spat from diuron-exposed genitors, a significant decrease in nuclear DNA content was measured, with over 15% of individuals showing DNA hypodiploidy. In this study, fluorescent *in situ* hybridization (FISH) was used to further characterize at the chromosomal level this diuron-induced DNA damage. rDNA genes (5S and 18-5.8-28S), previously mapped onto *C. gigas* chromosomes 4, 5 and 10, were used as probes onto interphase nuclei preparations. Results conclusively show higher aneuploidy (hypo- or hyperdiploidy) level in embryos from diuron-exposed genitors with damages on the three studied chromosomal regions. This work suggests that sexually developing oysters are vulnerable to diuron exposure, leading to negative impact on reproductive success and recruitment of oyster.

9

Single and mixture effects of selected emerging pollutants studied in Precision-cut liver slices of Atlantic cod (Gadus morhua)

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Pollutants affect aquatic life in many different ways, causing toxicity, disrupting the reproduction and development, and reducing the survival of organisms. Some mechanisms of action of certain pollutants have already been assessed, but one of the greatest emerging challenges is to understand the effects of mixture toxicity. The Atlantic cod (*Gadus morhua*) is an economically important species widely distributed in the North Atlantic, which is vulnerable to discharges from human activities, such as coastal industries and expanding offshore activities. In fact, adverse effects of discharges on cod reproduction and health, e.g. by disrupting endocrine signalling pathways have been demonstrated. The liver is the organ mainly responsible for metabolizing xenobiotic compounds through the biotransformation pathway and that makes it an attractive model for toxicological studies in fish. Thus, in the present study, we aimed to apply the precision-cut liver slices (PCLS) method as a tool to test the effects of common pollutants of distinct chemical origin, such as chlorpyrifos, bis(2-ethylhexyl)phthalate (DEHP), perfluorooctanoid acid (PFOA) and 17α-ethynylestradiol (EE2), individually and in mixtures, on cod liver, using gene level biomarkers (*vtg, cyp1a, fabp, cyp7a1, cyp24a1, hmdh)*. No significant differences were observed in the transcription of any of the target genes when exposed to single chemicals. Only *vtg* and *fabp* genes were up-regulated when exposed to EE2. Similarly, this both genes were also up-regulated when exposed to mixtures. But in addition, transcription of *hmdh* and *cyp24a1* was significantly down-regulated in the mixtures. Additive effects of selected compounds in both tested mixtures were detected, in comparison to single exposure conditions. Previous studies showed that most genes altered by exposure to a certain single chemical, may not be useful for diagnostic chemical causation in a mixture exposure situation, where a “something from nothing”-effect could be observed. The application of cod PCLS for screening complex environmental samples was demonstrated as a valuable tool. Further works are necessary to improve the sensitivity of this bioassay, in order to implement its application in future marine environmental risk assessment scenarios. Keywords: PCLS, pollutants, mixtures, cod

10

Toxic pressure in Dutch estuarine and coastal waters by chemical stressors inhibiting photosynthesis of marine microalgae

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In recent years sources, types and levels of chemicals in estuarine and coastal waters have increased as a consequence of anthropogenic activities. These chemicals can affect primary production at the basis of the marine food chain. Chemicals stressors are hypothesized to disturb the photosynthesis of phytoplankton and, therefore, the carrying capacity of coastal and estuarine waters. In our study we identified and confirmed the main contributors to the toxic pressure on microalgae in Dutch estuarine and coastal waters and investigated the toxic pressure on marine microalgae of these compounds under environmental conditions. Effect-Directed Analysis was performed with passive samplers at three estuarine and coastal locations in The Netherlands. Passive sampler extracts were tested for toxicity on marine microalgae using the Pulse Amplitude Modulation (PAM) fluorescence assay. To reduce the complexity of the extracts micro-fractionation in 96 well plates was performed using an UPLC-UV system. Compounds were identified and confirmed using LC-ToF-MS. In addition, spot water samples were taken at the three study locations, extracted with solid phase extraction and the confirmed compounds were quantified with LC-ToF-MS. To determine the toxic pressure, measured concentrations in spot water samples and water concentrations from monitoring data were used and dose-response curves of the confirmed compounds were determined in the PAM assay. Concentrations from monitoring data showed that for the toxic pressure at different locations along the Dutch estuarine and coastal waters in 2012 a low risk is expected. However, concentrations can occasionally reach potential effect levels. Based on our results the identified and confirmed biocides contribute to the toxic pressure in Dutch estuarine and coastal waters. *Acknowledgement* - This project is financed by DELTARES, NL.

11

Impact of pollutants and climate change stressors on embryo-larval development of the Pacific oyster Crassostrea gigas in the Arcachon Bay

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The Pacific oyster, *Crassostrea gigas*, is the main cultivated species in the Arcachon bay. In recent years, problems of recruitment and mortality of juvenile or adult oysters occurred. These crises can be indicative of changes in Arcachon bay’s water quality. Previous studies have highlighted the presence of many molecules, with significant concentration in water, mainly herbicides, such as metolachlor and its metabolites, and "anti-fouling" compounds such as copper (Cu). Occurrence of these molecules in the Arcachon bay could explain some of the problems encountered. In the context of global climate change, the effects of rising temperatures and salinity changes could alter development and survival of embryos and larvae. This study aims to assess the combined effect of contaminants, temperature and salinity on the early growth phase of Pacific oyster larvae. Embryotoxicity bioassay has been used. Spawning was triggered by thermal stimulation of male and female oysters. Eggs were fertilized with sperm to obtain embryos. Oyster embryos were then exposed to concentrations of 1 $\mu g.L^{-1}$ (corresponding to environmental concentration) 10 and 50 $\mu g.L^{-1}$ of Cu. Metolachlor was tested at 10 $ng.L^{-1}$ (environmental concentration) 100 and 1000 $ng.L^{-1}$. During exposure, embryos were incubated at different temperatures (18 °C, 20 °C, 22 °C, 24 °C, 26 °C, 28 °C and 30 °C) and different salinities (23, 27, 30, 33 psu). After 24h, developmental abnormalities of D-larvae were analyzed. Salinities from 23 to 33 psu, which are currently encountered in the Arcachon Bay, did not affect normal development of embryos. However, environmental concentrations of Cu and metolachlor increased developmental abnormalities. In addition developmental abnormalities increased in a dose-dependent manner. Larval development was also affected by temperature. Below 24 °C, there was no development of D-larvae. Beyond 24 °C, especially at 30 °C, there was an increase in developmental abnormalities in control and contaminated conditions. No difference observed between control and contaminated condition suggested a predominance of temperature effect. The present study confirmed the high sensitivity of Pacific oyster embryos to heavy metals, pesticides and increasing temperatures. Toxicity threshold were in the range of concentrations currently measured in the Arcachon bay. High temperatures seemed to have more effect than contamination.

12

Gut passage of microplastics and bioavailability of co-contaminants associated with microplastics in organisms exposed via diet or aqueous phase

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Microplastics contaminate marine environments and can have various toxicants (termed here co-contaminants) ad/absorbed to them that can be transferred to organisms. The association of model co-contaminants 17α-ethinylestradiol and phenanthrene with polyethylene microspheres was evaluated by assessing changes in expression of biomarker genes [vitellogenin (*vtg*) and cytochrome p450 1A (*cyp1A*) respectively] in larval zebrafish *Danio rerio* exposed in the aqueous phase. Zebrafish were used as an analytical tool to identify changes in the associations of the co-contaminants during ad/absorption reactions with microplastics. Gut retention and the time required for microplastics to pass through the gut of *C. maenas* was investigated according to microplastic diameter (45-55 μm & 850-1000 μm) and compared to coarse sand control (1000-1230 μm). Crabs were fed particles embedded in a gelatine pellet and uneaten pellets and faecal material were collected and particles enumerated over 240 hours after exposure. At a phenanthrene concentration of 0.5 mg/L the mean expression of *cyp1A* was 3.3 relative fold induction (RFI) and the RFI was 2.2 (lower, but not significant) after 5 d mixing with microplastics. In contrast, the results of 17α-ethinylestradiol (1 μg/L) experiments indicated a significant and up to 70% lower *vtg* expression in presence of microplastics. We found that *C. maenas* ingested microplastics in gelatine pellets, but that ingestion was inversely related to particle size and crabs did not distinguish between coarse sand and microplastics. The majority of all particles ingested by crabs were egested within 48 h, and near 100% recovery of all ingested particles indicated that absorption of microplastics was minimal (not detectable). The time taken for ingested particles to be egested differed among particle types (i.e., coarse sand compared to microplastics). Mean gut retention time (GRT) required for 50% of particles to be egested (i.e., the GRT_{50}) was lowest for coarse sand and significantly higher for both sizes of microplastics. Association with microplastics was related to physicochemistry of co-contaminants. *C. maenas* ingested food with microplastics and increased gut retention time for microplastics compared to other particulates (i.e., sand) suggests greater potential for associated co-contaminants to be released into organisms.

Waste and Wastewater effluents: chemical and ecotoxicological characterisation (I)

13

Taking the Chemical Pulse of a Nation by Example of the U.S.A.

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Municipal wastewater treatment plants (WWTPs) are designed first and foremost to protect human populations and the ecosystems humanity relies on. Yet, they also offer additional functionality in serving as chemical observatories for the sewersheds they service. If data are compiled from a large enough and representative sample of WWTPs, it becomes possible to determine the chemical

mass flow of large cities, regions, states and even a nation as a whole. By example of the United States of America, a case study is provided, demonstrating the utility and informational power of chemical monitoring at WWTPs. Aside from observing chemical mass flow through society, monitoring at WWTPs also enable the identification and characterization of the behavior of established and emerging pollutants. Harmful chemicals, which travel through the treatment process without undergoing any appreciable degradation, are of particular concern. They may be discharged into surface waters during reclamation of treated effluent or find their way into terrestrial environments via applications of treated municipal sludge, the semi-solid by-product of wastewater treatment. In the U.S., sewage sludge is routinely applied on land for inexpensive disposal of this abundant, unwanted material that also features desirable constituents, such as a high content of nutrients and organic carbon. Wastewater-borne chemicals of concern covered in this study include traditional environmental pollutants, such as toxic organohalogens (polychlorinated biphenyls, brominated flame retardants, perfluorinated surfactants, etc.) as well as a large number of pharmaceuticals and personal care products (PPCPs), whose adverse impacts on human health and ecosystems are still emerging. Data from meta analyses are provided, informing on the occurrence, removal, biodegradability, persistence, and mass flux of organohalogens and emerging contaminants through WWTPs. Case studies show that mass balances on chemicals during municipal sewage treatment may serve as a powerful tool for identifying unsustainable chemistry and for studying chemical consumption and disposal patterns. Effective utilization of this information by regulatory agencies can aid in curtailing environmental pollution, thereby limiting adverse ecological and human health effects.

14

Fate of pharmaceuticals in urine treatment

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Urine has a large potential to be an effective fertiliser due to its rich nutrient content, specially in developing countries. Since the majority of the pharmaceuticals taken by humans are excreted via urine, major concerns over the recycling of urine nutrients arise from the uncertainty whether pharmaceuticals contained in the fertiliser could have adverse effects on the soil or food. Therefore, research on the pharmaceutical content and possible removal processes is important. Biological treatment of urine with nitrification is a recommended technique for the stabilisation of nitrogen in urine and the removal of easily degradable organic substances. In this study, selected pharmaceuticals were investigated during urine treatment to elucidate whether a pharmaceutical free treated urine can be achieved. Based on preliminary urine screening results in Durban (South Africa), a representative mixture of 12 pharmaceuticals was chosen for batch experiments under anaerobic condition to simulate storage and during urine treatment under nitrifying conditions. Furthermore, sorption to activated carbon as post-treatment was also evaluated. This study is embedded in the VUNA project (www.vuna.ch, financially supported by the Bill & Melinda Gates Foundation), where a dry sanitation system was developed and is installed in South Africa to recover nutrients from urine in small decentralized reactors to produce a valuable fertilizer, affordable for the poor, to promote entrepreneurship and to reduce pollution of water resources. The experiments show that long-term storage is not sufficient to substantially remove the amount of pharmaceuticals in urine. However, in aerobic biological processes, as they occur in a reactor for nitrification of urine, elimination of several pharmaceuticals was observed. Since the main purpose of nitrification is the stabilisation of nitrogen in urine and the removal of easily degradable organic substances, pharmaceutical removal is a beneficial side-effect. A post-treatment of the nitrification effluent by adsorption to powdered activated carbon could reduce the content of all pharmaceuticals to acceptable low levels.

15

Multivariate Workflow for the Screening of Nontarget Peaks from Biologically-Treated Wastewater

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It is known that transformation products (TPs) may be formed during biological treatement of wastewater (WW). Together lab studies and targeted screening offer a limited picture of the compounds in treated WW. In this project, samples were collected from the influent and effluent of an activated sludge treatment process and analyzed with liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). For data processing, a combined nontarget screening and multivariate analysis approach was applied to select peaks of possible TPs for structure elucidation. A validation of the workflow was also done. Two sets of 48-hr composites were collected from a WW treatment plant in Duebendorf, Switzerland. These sets of composites were analyzed separately and were further subdivided into a validation set of samples, which were spiked with parent and TPs, and an unspiked set, used for nontarget analysis. Samples were filtered and enriched with an off-line solid-phase extraction (SPE) cartridge containing the following three layers: reverse phase, ionic exchange, and activated carbon. Samples were measured with LC-HRMS using electrospray ionisation (ESI). Data processing of the validation set was done with Thermo QuantBrowser while data processing of the nontarget set was done with R statistical software. Semi-quantitative target screening of over 300 compounds was done with EnviMass. Results from the EnviMass screening showed that while many target compounds were removed, a number also showed persistance over the treatment. Analysis of the validation set showed that principle component analysis (PCA) was successful in distinguishing influent and effluent samples in the first principle component (PC1). Additionally, the loadings indicated that generally parent compounds were associated with the influent samples, while TPs with the effluent samples. Similar analysis was carried out with the nontarget data. The PC1 explained 60% of the variance and could separate influent and effluent. But selection of the peaks at this stage was still difficult; therefore further selection of peaks was done using cluster analysis, multiresponse permutation procedure (MRPP), and indicator species analysis. This workflow generated a list of 2867 peaks associated with the influent and 759 peaks associated with the effluent ($p < 0.01$). These peaks can then further be selected based on chemical information (*i.e.,* isotope and/or adducts) for structure elucidation and identification.

16

Removal of Parabens and Benzophenone-4 from wastewater and environmental impact assessment

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Parabens (PBs) are chemicals widely used as preservatives by personal care products, pharmaceutical and food industries. Sunscreens are also important chemicals in these industrial sectors. Most organic sunscreens are oils soluble; however, Benzophenone-4 (BP-4) is water soluble and is used in the products called “oil-free”, formulated to feel less oily. PBs are becoming increasingly controversial, because some studies have shown that low level exposure cause weak estrogenic activity at environmentally relevant concentrations [1]. As a consequence of its widespread use and its chemical stability and resistance to biodegradation, BP-4 is being increasingly detected in surface waters [2]. The BP-4 levels that are commonly found in surface waters are generally too low to cause acute toxicity, but chronic effects cannot be ruled out. Indeed, *in vitro* tests have shown that BP-4 has multiple endocrine disrupting activities in fish [3]. Data on the removal efficiency of these compounds in WWTPs is still scarce. The aim of the present study was to investigate the presence of the 4 most used PBs, i.e. *methyl-, propyl-, butyl- and benzylparaben* and the most water soluble benzophenone derivative, BP-4, in wastewaters of a network of 20 WWTPs located in Spain. Samples were analyzed by a fully automatic on-line SPE-HPLC-QqTRAP-MS/MS method. Results confirmed the wide presence of PBs in both matrices, being the highest concentrations found for propylparaben in influent samples. Removal efficiencies for this set of compounds were extremely high (90-100%). Similarly, the UV filter was observed in all wastewater samples. Its elimination was pretty variable depending on the WWTP, showing removal efficiencies in the range 8-91%. The relationship among its half-life time, removal rate and the WWTP hydraulic retention time will be discussed. The risk posed for the aquatic organisms exposed to the effluents discharge was estimated, and the eco-toxicological implications will be discussed in the presentation, as well. **References** [1] P. Bjerregaard, P.R. Hansen, J.K. Larsen, C. Erratico, H. Holbech, Enviorn. Toxicol. Chem. 27 (2008) 2387. [2] R. Rodil, J.B. Quintana, P. López-Mahía, D. Prada-Rodriguez. Anal. Chem 80 (2008) 1307. [3] P.Y. Kunz, H.F. Galicia, K. Fent, Toxicol. Sci. 90 (2006) 349. *Acknowledgements:* This work was funded by the Spanish Ministry of Economy and Competitiveness [project SCARCE (2010 CSD2009-00065)].

17

Advanced treatment for removal of micropollutants in municipal effluents by fungal laccases

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Pollution of water courses is generally caused by dyes, amines, phenols, heavy metals and surfactants as well as newly discovered contaminants as Endocrine Disrupting Chemicals (EDCs). Recently, the environmental concern rose since commonly used treatment processes are only partially effective towards these molecules, causing their progressive and constant accumulation into the environment (wastewaters, river waters and drinking water). A biological approach opens new scenarios for the treatment of wastewaters contaminated by micropollutants: enzymes are indeed able to degrade a wide spectrum of xenobiotics operating at various pH, temperatures and ionic strength. In the present study, a *Trametes pubescens* strain was opportunely stimulated in order to produce the necessary bulk enzymes. Thus the laccase-mediated treatment was used towards

model and real solutions containing recognized EDCs, pharmaceuticals, PCPs, pesticides, etc. A multishot stir bar sorptive extraction with targeted in-situ derivatisation and a gaschromatography-mass spectrometric was used for analytes detection. This analytical method represents an innovative approach, enabling the contemporary detection of several target analytes in real municipal wastewaters: the matrix effect of the samples was negligible and micropollutants were quantifies up to ppt level (ng/l). Laccases demonstrated to be strongly active towards different molecules in spiked samples, being able to degrade at least 19 EDCs, belonging to different chemical classes. In most of the cases, the minimal effective enzymatic concentration was lower than 50 U/l and the analytes initial concentration was halved within the first 24 h. Due to the expected reduction when laccase crude extract is added to a complex matrix (i.e. wastewaters) with variable pH and active microflora, higher enzyme concentration (100 U/l) were adopted for the treatment of real wastewater sample. Laccases degraded most of the detected compounds in a range of 50-96% within 24 h. Besides, the analytes enzymes-catalysed conversion was followed by a significant abatement of the ecotoxicity and the estrogenic activity by means of different model bioassays. Further studies are in progress to optimize the operative parameters (i.e. working pH, laccase concentration, enzyme immobilization), in order to enhance the stability and the efficiency of the laccase-mediated system.

18

Treatment of micropollutants in municipal wastewater using white-rot fungi

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Treatment of micropollutants such as pharmaceuticals and pesticides in municipal wastewater is challenging due to their very low concentrations (ng/l to μg/l), their relatively low biodegradability, and their different physico-chemical characteristics. One potential way to improve micropollutant biodegradation in wastewater treatment plant (WWTP) effluent is by using microorganisms such as white-rot fungi that produce powerful unspecific oxidative exo-enzymes (laccase, peroxidase) that are able to oxidize several micropollutants recalcitrant to bacterial degradation. The goals of this study were (i) to evaluate the ability of white-rot fungi to degrade pharmaceuticals and pesticides and (ii) to develop a fungal treatment which allows removal of a broad range of pollutants but also long-term survival of the fungus in the system. For the first objective, batch experiments with the white-rot fungus *Trametes versicolor* were conducted first in defined medium containing glucose and spiked with 10 mg/l of pollutant (naproxen, sulfamethoxazole or isoproturon), and then in real unsterile WWTP effluents (not spiked) with wheat straw as sole carbon and energy source. *T. versicolor* was able to remove over 90% of naproxen in less than 24 h, and sulfamethoxazole and isoproturon in less than 4 d in defined media spiked with these pollutants. In real unsterile biologically treated wastewater, *T. versicolor* could remove over 90% of the anti-inflammatory drugs diclofenac and mefenamic acid in less than 24 h, and naproxen in less than 48 h, despite their low concentrations (400-800 ng/l). These good removal rates were however not observed for other compounds that needed longer reaction times, due to the short survival (one week) of the fungus in unsterile wastewater. To increase the chance to maintain this organism in the treatment, we designed a fungal unsaturated biotrickling filter composed of wood chips colonized by the mycelium of the white-rot fungi *T. versicolor* or *Pleurotus ostreatus*. Unsterile tap water or treated wastewater spiked with pollutants (10 mg/l) were then trickled with recirculation through the wood-mycelium support. This system allowed a much better survival of the two fungi and was able to remove almost completely naproxen, diclofenac, mefenamic acid, paracetamol, bisphenol A, and triclosan in less than 8 h. Future experiments will show whether the oxidation potential of these fungal filters is applicable to a wider range of pollutants.

Fate and effects of nanoparticles under environmentally realistic conditions (I)

19

Nanomaterials in the aquatic environment: status and challenges ahead

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Nanotechnology is expanding rapidly, thus increasing the release of engineered nano-materials (NMs) to the aquatic environment. Sediment is likely to be the environmental compartment most exposed to NMs, and organisms that feed on or live in sediment are likely to be particularly at risk from NM exposure. Due to their increased surface area and reactivity, NMs may be more bioavailable and toxic than their macroscale counterparts. However, very little is known about the environmental impact of NMs and whether they behave similarly in the environment compared to their macroscale counterparts. These factors make it difficult to perform environmental risk assessments and to set quality standards for NMs. Some of the questions we are facing are whether ***nano-specific properties lead to unexpected and new biological effects? If so, is the potential for such effects diminished when NMs are introduced to a complex environment such as the sediment compartment?*** The US-EU Community of Research (CoRs) was established in 2012 to provide a platform for scientists to develop a shared repertoire of protocols and methods to overcome nanoEHS research gaps and barriers. The overall goal of the Ecotoxicity Testing and Predictive Modeling CoR (Ecotox CoR) is to encourage the evolution of i) hazard assessment methods and predictive models built on the foundations of fundamental research characterizing fate (including ageing) of nanomaterials in different environmental compartments and the interactions of nanomaterials with biota and ecosystems, and ii) communication among regulators, experimentalists, modellers e.g., to make data available/presented in a useful data format to help modellers, experimentalists and risk assessors. Based on ongoing work in the Ecotox CoR and two Ecotox CoR workshops we here provide an overview of the state-of-the-art of NMs in the aquatic environment and discuss the challenges ahead by providing suggestions for future research needs that will enable us to perform sound risk assessment and set quality criteria for NMs.

20

Engineered nanoparticles interactions with the environment: towards an understanding of the risk they pose (ENPERA): realism Vs regulation

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At present, there is a worldwide focus on engineered nanoparicles (ENPs) in the environment, the possible risks they pose and the potential need for nanospecific environmental risk assessment and legislation. The ENPERA project (Engineered nanoparticles interactions with the environment: towards an understanding of the risk they pose) specifically targets engineered nanoparticles and how they influence “system and process understanding” and “what are the effects of distribution of NPs and how can these be dealt with”. The project has focussed on establishing a knowledgebase that will inform the process of understanding the risks posed to the environment from ENPs, specifically focusing on environmentally relevant exposure scenarios and consideration of fate and effects of ENPs in the environment and within target organisms. Over the past 3 years we have undertaken several projects under the auspices of ENPERA looking at varying environmentally factors and conducting exposure scenarios that best reflect the natural environment (e.g. varying environmental parameters, mixture toxicity with other contaminants of concern in the environment). Another important part of this project has been the involvement in risk assessment and regulation of nanomaterials and the difficulties of coupling both a realistic evaluation and best reflecting the future regulatory requirements for nanomaterials. This presentation summarises the results of the 3 year project and presents a position on the future direction of the assessment and regulation of nanomaterials in the aquatic environment.

21

Modelling fate and transport of engineered nanoparticles in the aquatic environment

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Nanoparticles in the aquatic environment are subject to a variety of\nprocesses, such as aggregation to suspended matter and subsequent\nsedimentation or dissolution and chemical transformation. Several\nresearch groups have reported the development of the concentration of\nnanoparticles over time as a result of these processes. This contribution presents a mathematical model for the behaviour of\nnanoparticles in the aquatic environment based on laboratory experiments\ndescribed in the literature. In these experiments the aggregation and\nsedimentation of nanoparticles in water were investigated using different\ntypes of water and different initial concentrations. The mathematical model describes such processes as homoaggregation\n(clustering) and heteroaggregation (adsorption to suspended matter)\nand sedimentation by accounting for various fractions.\nAfter calibrating the model, it was able to adequately describe the\nexperimental data that covered a wide range of initial concentrations.\nUsing the model it is possible to determine which of the processes have\nmost influence on the nanoparticles. For instance, homoaggregation and sedimentation of the clusters of\nnanoparticles thus formed turn out to be the dominant processes if the\nconcentration in the water is of the order of 10 mg/l or more. Only when\nthe concentration is much lower, aggregation (adsorption) to suspended\nmatter becomes appreciable. More experimental datasets are required, however, to determine if the\nprocess parameters that were found here are generally applicable and to\nrefine the model.

22

Spatially explicit fate modelling of engineered nanoparticles

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Spatially explicit modeling approaches describing the fate of engineered nanoparticles (ENPs) are crucial to advance the site-specific risk assessment of ENPs. Here we introduce a spatially and temporally explicit aquatic fate model for ENPs. This allows for linking ENP fate processes to key hydrological characteristics, e.g. resuspension is linked to the flow rate and aggregation rates are related to the shear stress, which in turn are governed by flow rate. The model further accounts for advective transport, homo- and hetero-aggregation, sedimentation-resuspension, particle degradation, dissolution and burial. Because these processes greatly depend on local conditions, they need to be taken into account for the assessment of site specific risks. Five ENP size classes and naturally suspended solids (NSSs) are considered, returning 25 different hetero-aggregation interactions among different size classes of ENPs and NSSs. Spatially explicit scenario studies were performed for the Dutch river Dommel. Spatially heterogeneous versus uniform scenarios, different emission scenarios and different ENP-type scenarios are compared. As an example outcome: modeling with spatially uniform conditions predict that 99% of the ENPs reach the end of the canal. In the same scenario using the actual characteristics of the Dommel catchment, only 30% of ENP heteroaggregates reach the end of the modeled stretch. This illustrates how important spatially explicit modelling is of site specific exposure- and risk assessment of ENPs.

23

Transformations of silver nanoparticles relevant to product use: exposure to disinfectants and washing solutions

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In the context of assessing the potential risks of nanomaterials (NMs), a life cycle approach (covering production, use and disposal of the NM or nano-product) can represent a holistic view of their potential impacts. Considering that the NM life cycle is determined by their application within a product, relevant exposure scenarios and particle aging/transformations are strongly dependent on the life cycle of nano-enhanced products themselves. For example, treated surfaces will be subjected to oxidants during disinfection and cleaning while nanomaterials embedded in textiles would transform after exposure to detergents during washing. Here, we simulated the aging of Ag engineered nanoparticles (ENP) of different sizes (20 and 40 nm) and surface chemistries (uncoated, citrate, PEG and fulvic acid) through storage, exposure to oxidants (H_2O_2 ranging from 0.03% to 3%) and/or to suspension in standard detergent formulations. Transformations over multiple exposures (e.g. aged suspension in detergent and subsequent exposure to oxidants) were also investigated. Observations of transformation to Ag ENPs from the various physical and chemical treatments included measurement of particle dissolution (Ag^+ release, primary particle diameter reduction), characterization of surface transformation(s) and precipitation of “new” Ag NP from Ag^+. All particles that experienced product-use relevant ageing underwent transformations that changed their fundamental starting characteristics. Furthermore, exposure of a particle to one treatment greatly affected the rate and extent of further transformation(s) upon a second treatment step. This work highlights the fact that since Ag ENPs will readily be aged/transformed during a products life cycle and it is be prudent to consider how these by-products differ from pristine ENPs. This is especially true when attributing toxicity or safety assessments to materials, which may be best done to the (relevantly) transformed particles rather than to the pristine version.

Human exposure to emerging contaminants: monitoring and modeling (I)

24

Does foam application reduce aerosol formation?

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Foam spraying is often used as an alternative to droplet spraying for the treatment of surfaces. It is generally assumed that aerosol exposure is considerably smaller for foam sprays compared to conventional droplet mists. This especially refers to the application of biocides in the occupational section, e.g. the disinfection in the food industry as well as in the consumer section, especially for cosmetics and household cleaning agents (“convenience products”). While there is a well-developed quantitative understanding on the physical and technical parameters determining the exposure risk when using droplet sprays, the characterization of potential aerosol exposure for the application of foams has received little attention so far. In this study the current state of knowledge on the use of foam sprays for biocide application was surveyed from the literature. In addition some exploratory aerosol measurements were carried out for typical biocide products to compare aerosol release from foam and droplet spraying. More than 55 biocidal substances, such as QAV or peracidic products as well as pyrethrins and courmarins comprising products for disinfection, insecticides and rodenticides, are used in foams. The foaming technologies can be classified as mechanical foam generation using low and high pressure and blowing agent foam generation. Since no information on aerosol formation during foam spraying was found in the literature, exploratory aerosol measurements were performed in a laboratory set-up. A reduction of a factor of three was determined for aerosol release in the three health-related size fractions (respirable, thoracic, inhalable), when the biocidal product was sprayed as a foam compared to droplet spraying. Generation of semi-solid, highly-viscous foams using physical blowing agents lowered aerosol formation more than hundred fold. The experiments show that aerosol exposure cannot generally be ruled out when using foams but increasing foam viscosity seems to reduce aerosol release. Thus, further investigations should specify to what extent foam could be explained by physical chemical data of biocidal products by using specific application techniques. These factor determine not only the primary aerosol inhalation exposure but also the risk for exposure and mobility of the environment. Correlations allowing for a prediction of aerosol release from process and product parameters have to be established in future more comprehensive experiments.

25

Sorption of SVOCs to fabrics: Towards determining the role of clothing in human exposure

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The indoor environment, with its limited air exchange rate and degradation pathways, holds a large inventory of products and materials that contain SVOCs like flame retardants and plasticizers. These chemicals are released into air by degassing followed by partitioning into dust according to their physical-chemical properties (Shoeib et al, 2012). We hypothesize that clothing acts as a platform for the deposition and re-suspension of particle-phase SVOCs and as a sorbent for the gas phase. To test this hypothesis, studies were conducted to determine the difference in sorption behaviour of cotton and polyester for SVOCs in chamber experiment and to study the sorption of SVOCs from ambient indoor air to pre-cleaned fabrics deployed in homes. The results from chamber experiment suggest that uptake of SVOCs by polyester is faster than cotton, but over time cotton sorbs more because of its large micro-surface area. Also, SEM images of cotton showed its large micro surface area which would provide more area for sorption in contrast to polyester. In case of the fabrics deployed in homes, cotton and rayon showed sorption of mainly BDE-209 along with some BDE-47 and DBDPE in living rooms whereas, those deployed in the bedrooms were dominated by the congeners of penta-BDE that is used in flexible foam furniture. Cotton and rayon which is "regenerated" cellulose fabric (Shaikh et al, 2012), showed similar sorptive behaviour. This study provides evidence for the sorption of gas-phase SVOCs to fabrics under controlled and ambient conditions. Sorption is influenced by the physical-chemical properties of SVOCs (as expected) and fabrics, as well as the morphology of the fabrics.

26

Lead and mercury levels in cord and maternal blood and placental tissue among Korean population

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Heavy metals are naturally occurring and can cause serious adverse effects especially during early fetal development. Lead and mercury have long been suspected for various health effects ranging from endocrine disruption to neurodevelopment. This study was conducted to determine the levels of lead and mercury among sensitive human populations including pregnant women and newborn infants. The levels of both metals were also determined in placental tissue. For this purpose, a total of 105 pairs of pregnant women and their matching infants were recruited from four university hospitals during 2011 and 2012. Blood samples were withdrawn from pregnant women a day before delivery. Cord blood and placental tissue were collected during delivery. Samples were stored at -70 oC until analysis. Auto mercury analyzer (SP-3DS, Nippon) and Graphite Furnace Atomic Absorption Spectrometer (AA6800, Shimadzu) were used for the analysis of merucury and lead, respectively. Lead and mercury were detected in 98.7%, and 100% of the samples, respectively. Lead was detected in the maternal blood, placenta and cord blood 2.62±1.14 ug/dL, 6.85±7.93 ng/g, and 1.91±1.18 ug/dL, respectively. Mercury was detected at 4.83±2.45 ug/L, 9.82±5.48 ng/g, and 7.84±3.55ug/L, respectively. The concentrations of the heavy metals in placenta were significantly correlated with those in maternal bloods as well as in cord blood. The placental tissue contains about 2-3 times greater concentrations of lead and

mercury compared to maternal blood. For mercury, the levels among three types of biological samples showed significant correlations ($p < 0.01$), however for lead significant correlation was only detected between maternal and cord blood, suggesting different kinetics in the pregnant women. Further studies on sources of exposure to these metals and their potential health consequences are warranted.
Keywords Metal, placenta, transfer, newborn

27

Human exposure to UV filters in personal care products

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Organic ultraviolet (UV) filters are designed to protect our skin from UV-induced damage. Apart from sunscreens, they are found in many other categories of personal care products (PCPs) that are often concurrently used by the same consumer. The potential endocrine-disrupting effects attributed to organic UV filter ethylhexyl methoxycinnamate (EHMC) are being debated. EHMC can be systematically absorbed through the skin from PCPs, therefore aggregate exposure to EHMC from the use of multiple PCPs and the relative contribution of different PCP categories to the aggregate exposure need to be determined to enable concerned consumers to take steps to reduce their exposure. PCP use and co-use patterns and EHMC concentration data are key input variables to our exposure model. To date, publicly available PCP use and co-use patterns are limited. Moreover, in Europe, EHMC concentrations in PCPs are kept confidential. Therefore, in this study, PCP use and co-use patterns were determined by a postal questionnaire in the German-speaking part of Switzerland (1196 respondents), which included, for the first time in Europe, very young consumers, who may be more vulnerable to adverse effects attributed to endocrine disruptors. EHMC concentrations were measured in 116 PCPs that were frequently used by the survey respondents. In general, EHMC concentrations in PCPs applied regularly throughout the year are similar or higher than those in sunscreens that are used for sun protection and applied only on selected days. For each respondent, the aggregate exposure was estimated at an individual level by combining the self-reported PCP use patterns with the EHMC concentrations measured for the self-reported products. Overall, Swiss-German consumers are selecting sun-protection products in their daily lives as shown by the highest relative contribution to EHMC internal aggregate exposure levels from the use of face cream. Detailed distributions of internal aggregate exposure levels of EHMC along with the human health risk characterisation will be presented at the conference. Our study also highlights the urgent need to determine both EHMC metabolism in humans and its transdermal penetration.

28

Integrated External and Internal Exposure to Chemicals: the INTEGRA method

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INTEGRA brings together all available information within a coherent methodological framework for assessing the source-to-dose continuum for the entire life cycle of substances covering an extensive chemical space. The major component of INTEGRA is a unified computational platform that integrates environmental fate, exposure and internal dose dynamically in time, including also inverse modeling for exposure reconstruction and HBM data assimilation. The INTEGRA method was applied on assessing exposure to bis(2-ethylhexyl)phthalate (DEHP). The aim of the study was the identification of potential exposure scenarios (or combinations) among intended uses of DEHP and how these are translated into internal exposure, as well as they are reflected into biomonitoring data. The magnitude of exposure scenarios was estimated based on extensive literature review in DEHP manufacturing and processes for intended uses, residues in food and existing biomonitoring data. Intended uses of DEHP include building and construction materials, car interior (vinyl upholstery), clothing (footwear, raincoats), food packaging, children's products (toys, grip bumpers), gloves, medical devices, PCPs and cosmetics. Scenarios related to the overall cycle assessment of DEHP in building materials were of interest. The overall life cycle assessment of vinyl flooring manufacturing showed that the environmental contribution to the total exposure to DEHP is negligible. Exposure to DEHP is attributed to vinyl flooring emissions. In a residential dwelling (surface area of 270 m^2 and air exchange rate of 0.5 h/r) characterized by DEHP gaseous emissions of 200 µg/h (vinyl flooring and other plastic materials), the concentrations of DEHP in the gaseous, particles and dust phase are equal to 1.5 $\mu g/m^3$, 21 $\mu g/m^3$ and 4400 µg/g settled dust. Overall daily intake varies between 0.2 to 10 µg/kg-bw, depending on the exposure scenarios considered. The latter are age-dependent: adults are exposed mostly through inhalation and infants through non-dietary ingestion. For a common repeated aggregate exposure scenario of 2 µg/kg-bw, the DEHP internal dose in venous blood and in adipose tissue (where bioaccumulation is clearly observed) reach a quasi-state equilibrium of 0.07 and 0.4 µg/L respectively. The expected urinary concentrations of MEHP, 5-OH MEHP and 5oxo-MEHP are 3.1, 16 and 8 µg/gCr respectively. The modeled data agree nicely with extensive HBM data from the USA and Europe.

Advancing Adverse Outcome Pathways for Integrated Toxicology and Regulatory Applications (I)

29

Advancing Adverse Outcome Pathways for Integrated Toxicology and Regulatory Applications

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Recent regulatory efforts in many countries have focused on a toxicological pathway-based vision for human health assessments relying on *in vitro* systems and predictive models to generate the toxicological data needed to evaluate chemical hazard. A pathway-based vision is equally applicable to ecological risk assessment. Pathway-based analysis of chemical effects opens numerous opportunities to apply non-traditional approaches for understanding the risks of chemical exposure. Similarities in molecular initiating events and key events that lead to toxicological outcomes provide a defensible framework for extrapolating chemical effects across species, even if the specific adverse outcomes differ between species. This opens the door for much more integrated approaches to chemical hazard evaluation in support of either human health or ecological risk assessment, that are based on consideration of pathway-conservation rather than the taxonomic origin of the text system. The objective is to maximize the predictive utility of available information and those data that can be generated most efficiently and cost effectively. However, these promising concepts and approaches for using pathway-based data in hazard screening and risk assessment need further development in order to realize their full potential. This talk will be an overview of the outcomes from an international workshop held in Somma Lombardo (Italy) in March 2014 exploring how to advance the use of AOPs for integrated toxicology and regulatory applications.

30

OECD Activities on Adverse Outcome Pathways

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1. Introduction Developing An Adverse Outcome Pathways (AOPs) requires gathering and organising large amounts of information from molecular initiating events to toxicological effects, which can only be achieved through international and interdisciprinary cooperation. In 2012, the Organisation for Economic Co-operation and Development (OECD) launched a new programme on the development of AOPs. 2. OECD AOP Development Programme The OECD published guidance on developing and assessing AOPs in 2013. It provides an insight into which pieces of information are necessary to identify and document an AOP and how to present them, and provides initial assistance on how to undertake the assessment of an AOP. A template has been included allowing authors to develop thorough AOPs and to improve consistency in AOPs. The OECD published the first AOP for skin sensitisation in 2012, describing the state of knowledge of the AOP for skin sensitisation initiated by covalent binding to proteins, assessing the weight-of-evidence supporting the AOP, identifying the key events. A format for an AOP project proposal has been developed. As of November 2013, more than 20 projects have been includeded in the programme, of which 17 are AOP development projects, three are case studies, one is a knowledge management tool, and one other project. 3. Process for the Development of AOPs at OECD The proposed process can largely be categorized into three phases: 1) submission of a project proposal, 2) development phase, and 3) endorsement phase for a publication. Most of the development and review is intended to take place via a web-based IT tool, AOP wiki, which is in development and testing. The Wiki provides developers the necessary steps to capture the scientific information in a user-friendly manner. 4. Conclusions and Next Steps The OECD, supported by its members and other stakeholders, is actively preparing a comprehensive framework as well as guidance, the IT platform and the process for developing AOPs. The potential issues to be considered are: (1) how to link the quantitative information from a key event to a key event in a meaningful way; (2) how to use the AOP concept to harmonise national integrated approaches to testing and assessment, thereby decreasing reliance on animal testing; and (3) how to build confidence that AOPs are also useful for negative predictions.

31

Using Adverse Outcome Pathways for regulatory applications

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In principle, AOPs offer a scientifically-credible foundation that supports greater use of predictive, mechanistic, data concerning the initiation or progression of a toxic insult for hazard assessment and regulatory decision-making. However, in practice, before the predictive relationships represented as AOPs are likely to be widely used for regulatory applications, there is a need for the scientific and regulatory community to indicate "acceptance" of those relationships. This involves generating confidence by showing that the relationships represented are consistent and reproducible and understanding mitigating factors and variables which can influence the outcome(s) or contribute uncertainties to the AOP-based extrapolation or predictions. Recognizing that different types of regulatory decisions require different levels of confidence or certainty, it also involves understanding the relative levels of uncertainty that make an AOP fit- or unfit-for-purpose, for a given regulatory application. This concept of AOP "acceptance" is incorporated into OECD's process for the development of AOPs (http://www.oecd.org/env/ehs/testing/moleculars creeningandtoxicogenomics.htm) . Specifically, it has been proposed that once an AOP description document has been developed by a stakeholder group or organization, those AOPs would be reviewed by OECD expert groups, and revised as necessary, before endorsement by the WNT (National Coordinators on Test Guidelines) and the Task Force on Hazard Assessment. However, the details of how AOPs would be reviewed, how suitability-for-purpose for different regulatory applications would be considered in applying AOPs, and how they would be used in quantitatively predicting hazard or risk due to activation of an AOP remains unclear. As part of the international workshop "Advancing Adverse Outcome Pathways for Integrated Toxicology and Regulatory Applications" held in Somma Lombardo, Italy a group of experts from academia, government and industry selected a set of well-developed AOP descriptions to serve as examples to examine how AOPs could be approved for use in a regulatory application. Case examples were developed using existing data for chemicals in that act through the AOPs. The ability to quantitatively estimate hazards and the uncertainty associated with different levels of pathway information present for an AOP were also assessed. Finally, recomendations were given as to how AOPs can be used in a regulatory context.

32

Use of weight of evidence for characterizing adverse outcome pathways in risk assessment

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Information and data on chemicals from studies published in the open literature are increasingly being used for assessment purposes by regulatory agencies in many jurisdictions, including North America and Europe. As most of these studies are not conducted to the Good Laboratory Practices (GLP) standards as required by regulatory agencies, there is a need to assess their quality and relevance in light of the regulatory endpoints being considered. These data need to be integrated into lines of evidence that inform adverse outcome pathways (AOPs) and lines of evidence related to apical endpoints such as survival, growth, development, reproduction. Specifically, the strength of the experimental methods, and the ecological relevance of the observed responses were scored. The strength of the methods was scored based on various aspects, such as the experimental design and conduct, the use of appropriate controls, measures of exposures, the inclusion of environmentally realistic concentrations, number of concentrations, quality control, and transparency of data. The relevance of the each response was assessed by scoring statistical significance, concentration or dose-response, its relevance to an appropriate apical endpoint, and a biologically plausible mechanism. The WoE process was inclusive and no studies were excluded, except those with mixtures where the individual components were not tested individually. Results were presented graphically for easy interpretation. Where responses are relevant, understanding or explanation of the mechanisms at lower levels of organization is possible. This might facilitate extrapolation to other organisms/taxa or identify reliable and robust biomarkers that can be used in place of the apical endpoint. If one or more apical endpoints have been assessed under WoE, and the combination of these indicates no or *de minimis* effects, an analysis of AOPs is not needed. However, if one or more of the apical endpoints indicates relevant effects, then a characterization of AOP might be useful to better understand the response. The need for WoE and an illustration its application will be presented with the herbicide atrazine as the case-example.

33

Development of an alternative testing strategy for the Fish Early Life-Stage (FELS) test using the AOP framework

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Currently, the Fish Early Life-Stage (FELS) test (OECD 210) is the primary guideline used to estimate chronic toxicity of regulated chemicals to fish. Both industry and regulatory institutions have recently expressed their interest in developing an alternative testing strategy for the FELS test with specific focus on non-animal alternatives and including mechanistic information. Our lab recently started a project funded by CEFIC (LRI-ECO20-UA) that uses the adverse outcome pathway (AOP) framework to classify chemicals according to the toxicity mechanism and predict chronic adverse outcomes based on early events. We propose a combination of refined 5 day ZebraFish Embryo Toxicity (ZFET) tests and *in vitro* tests to study events at lower levels of biological organization along the AOPs and investigate the predictivity of these events for FELS toxicity. Exchanging data between toxicological and ecotoxicological studies offers the potential to find new clues to develop AOPs. Here, we present a case study in which we use *in vitro* data from the US EPA ToxCast™ program to support ecotoxicological risk assessment. We hypothesize that specifically acting chemicals (e.g. pesticides) also induce baseline toxicity (non-polar narcosis) depending on their octanol-water partition coefficient ($\log K_{ow}$). The goal of this case study is to use the ToxCast™ dataset to investigate which endpoints are dependent on the $\log K_{ow}$, reflecting a high probability that they are linked to the narcosis mechanism. We identified 49 ToxCast phase I *in vitro* assays in which the probability of a hit increased with increasing $\log K_{ow}$ of the chemical. Apart from assays measuring general cytotoxicity, 36 of these assays reported gene expression changes relevant for different biological processes including a.o. drug metabolization and transport, immunity and cell migration. In a next step, we will translate these *in vitro* assay endpoints to *in vivo* QPCR targets to include as endpoints in the 5 day ZFET test to investigate their potential in predicting chronic toxicity of non-polar narcotics. Although the selected endpoints are often referred to as aspecific, they are probably most important for predicting chronic toxicity through narcosis. An important aspect in risk assessment of chemicals remains to identify which AOP will most likely dominate the adverse outcome.

34

Development of Adverse Outcome Pathways for Endocrine Disruption in Daphnia Magna

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A number of exogenous compounds have the potential to interfere with the endocrine system of animals and may perturb vital endocrine processes to a degree causing an adverse effect (outcome) on ecologically relevant endpoints such as growth, development and reproduction. These endocrine disrupting (ED) effects have been well characterized in aquatic vertebrates and mammals due to a well-defined endocrine system and substantial research effort in the last decades, but knowledge on ED effects in a larger range of species are still poorly characterized. Lack of knowledge on ED effects in invertebrates is currently a major limiting factor to properly perform risk assessment of endocrine disrupting chemicals (EDCs) across taxa. The present project aims to develop and evaluate adverse outcome pathways (AOPs) for ED in the freshwater crustacean *Daphnia magna* by linking responses at the molecular level with adverse outcomes (AOs) relevant for regulatory processes. A literature survey was performed first to collect existing knowledge of ED effects on *D. magna* or closely related crustaceans.

Putative AOPs were assembeled to identify knowledge gaps prior to experimental studies. Computational and experimental methods were additionally used to identify possible ED targets in *D. magna* and discover novel EDCs in crustaceans for subsequent verification of potential mode of action (MoA). I*n vivo* exposure studies were then conducted with standardized test protocols, modified to accommodate sampling and toxicogenomics analysis, functional and apical toxicity endpoints that potentially perturb key molecular inititating events (MiEs), key events (KEs) and AOs of regulatory relevance. A custom *D.magna* oligoarray was deveploped to study the stress-responses at the transcriptomic level and to characterize potential MoAs of selected EDCs. In these experimetnal studies, juvenile *D. magna* were exposed to the endogenous crustaceans hormones methyl farnesoate and 20-hydroxyecdysone and the two pesticides fenoxycarb and fenarimol that are known to interfere with the normal function of the endocrine system in *D. magna*. Results from chemica exposure were compared with the reference hormones to obtain relative efficacy and potency of their ED effects. More in-depth characterization of the ED MoAs were obtained by transcriptomic analysis. The present project used several advanced tools to link the MiEs to adversity in *D. magna* and may inform future hazard and risk assessment of EDCs.

Modelling techniques for future-oriented LCA and forecasting scenarios (I)

35

How to define future LCA scenarios addressing the effect of climate change in crop production

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When LCA is intended to be used to affect strategic long term decisions, the challenge of defining future systems with many uncertain and unknown aspects needs to be faced. One important contribution is provided by scenario analysis. This paper elaborates on this issue, addressing scenario analysis in LCA with regard to one of the most debated issues in the climate change debate, i.e. climate change effect on primary agricultural production. Considering the case study of future barley production in a changing climate we aim to address the following question: how can future scenarios be performed in LCA when addressing climate change effects on crop production? We performed a cradle to farm gate study of 1 kg dry matter spring barley for malting in Denmark, focusing the definition of scenarios at the life cycle inventory level through a three steps-approach: (1) definition of the current baseline scenario and hot-spots analysis; (2) identification of the main deviations from the current production based on the best available data and knowledge; (3) suggestion of adaptive measures to compensate for possible changes due to the changing climate. Results show that priority in the definition of future adaptive measures should be put on crop yield. Data on crop yields come from cultivation experiment where the performance of the crop is investigated under the predicted climatic conditions. These data are therefore associated with relatively small uncertainty. On the contrary, the estimation of other parameters, such as pesticide treatment index and leaching from fertilizer application is affected by many unknown factors and should be addressed with sensitivity analysis. The definition of the range of variability of sensitive parameters should be based on best estimate from expert judgements from breeders and farmers. Furthermore, when presenting the results of a multiple scenario study focusing on different adaptive measures, the issue of uncertainties included in each of the scenarios should be addressed by parameter uncertainty analysis. The proposed approach can be implemented using the tools already included in the interpretation step, i.e contribution analysis, sensitivity analysis and uncertainty analysis. The output of long term scenarios based on LCA results is only effective in informing policy makers, if criteria to detect sensible aspects are taken into account, then the risk of defining unrealistic scenarios is minimized.

36

Modeling technique for territorial LCA applied to urban water systems: evaluation of prospective scenarios in megacities

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There is a need for future-oriented LCA and forecasting scenarios assessment for managers of water systems in big cities, mainly because of water scarcity and population growth. Urban water systems environmental performance have already been assessed with LCA focusing only on the technological part of the systems. New LCI and LCIA developments regarding water use impacts and territorial assessment are of great importance to increase the relevence of urban water systems LCA. The objective of this study is to develop a generic model for the environmental evaluation of prospective scenario of urban water systems. The model is developed at a city scale combining impact assessment with the calculation of provided services following the framework of territorial LCA. An urban water system includes three types of subcomponents: technologies (drinking water production, waste water treatment, etc), users (domestic, industrial, etc) and water resources. The proposed model allows the inter operation of these types of subcomponents in an integrated approach. For that purpose a generic subcomponent model that can be customized is established. From the combination of the different subcomponents that defines a scenario, direct water flows (quantity and quality) entering and leaving the system are directly modeled. Background LCIs concerning energy, chemicals and infrastructures of technologies and services provided to users are generated from litterature. Impact assessment phase takes into account new methods for water use impact. The model scales have been chosen to address water management stakeholders main issues: (i) temporal scale should allow differentiating the seasonal effect on water scarcity (ii) the spatial scale should discriminate at the sub-river basin scale the cascade effects on downstream users. Impacts of background processes are assessed with generic impact assessment methods. The model provides indicators at midpoint or endpoint level as well as water footprint. The model is first applied to the actual urban water system of the Paris suburbs area. Then several different scenarios coupling management options are defined and modeled based on the combination of the subcomponents. It demonstrates the applicability of the model: the creation of models for various scenarios is quite simple and the LCI effort is reasonable. Results are analyzed for the evaluation of the urban water system giving information for eco-design, planning and decision-making.

37

Using time-dynamic LCA and probabilistic analysis for prediction of future impacts

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In the last 50 years new technologies and social developments change the world drastically. Due to a more and more rapidly changing and evolving world the environmental assessment and rating of future technologies, uncertainties and decision become more and more important. Currently, LCA is a static methodology related to boundary conditions of a certain time and based on evaluations done for a certain time period. Especially uncertainty in future decisions is so far not assessed. Decisions made in the present are supposed to be carried out the same way in the future. At best several static LCAs with different scenarios are conducted to show the range of possible results. All of this is not yet sufficient due to the large range of possible results and not captured changes of future events (e.g. technology, regulations, etc.). In order to transparently and consistently assess these previously mentioned future events a time-dynamic, probabilistic Life Cycle Assessment has to be conducted for LCA studies with a long timescale, rapidly changing technologies or a possible change in usage. Using time-dynamic parameterized LCI-models by integrating time related dynamic changes in physical or technical systems leads to more significant LCA results. Therefor a time-dynamic, probabilistic LCA study not only foresees changes regarding the LCA datasets (e.g. electricity mix) - that can be assessed by means of a time-dynamic LCA - but also future uncertainties in the decision process can be implemented with the help of probabilistic representation (e.g. decision trees). As a result not only can future tendencies be assessed but also a range can be given for the uncertainty of future impacts due to the possible future changes. Moreover sensitivity analysis can be applied to check the robustness of the final results. By means of this approach the final model can also be used for later monitoring of future events and the results can be adapted on the latest occurring circumstances. This can be important for companies that set themselves an environmental goal for their product and want to assess how probable a compliance of that goal is. Therefore decision makers can dispose based on more reliable, consistent and transparent results. All in all time-dynamic, probabilistic Life Cycle Assessment could help to better understand the effect of future technologies and support decision makers today.

38

Assessing the prospective environmental impacts of photovoltaic systems based on a simplified LCA model

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The increasing electricity demand and the limited fossil energy resources require the development of new energy policies, based on more environment-friendly electricity-production technologies. Assessing the environmental impacts of these technologies is thus a central issue to design the future electricity mix. This can be done using life cycle analysis (LCA). However, LCA requires the collection of a large amount of data and is thus time-consuming. Besides, LCA results found in the literature related to one energy pathway show a large variability, reflecting the heterogeneity of systems and their modeling within this pathway. To address these issues, Padey *et al.* (2013) developed a generic methodology, based on the definition of a simplified parameterized model estimating the environmental performances of a set of systems as a function of their key parameters. This methodology first characterizes the environmental profile of one energy pathway, by setting a reference LCA model. The latter generates the environmental performances distribution, representative of the potential configurations for the

studied pathway, by taking into account the different sources of variability (technological, geographical …). The ones explaining most of the performances variability are identified as key parameters using global sensitivity analysis. A simplified model function of these key parameters is then defined to estimate the environmental performances of the studied pathway. In this study we apply this methodology to the photovoltaic (PV) energy pathway for monocristalline, polycrystalline, and thin-film technologies, and extend it to assess their prospective environmental impacts. More precisely, the methodology is adapted to investigate whether possible future changes in the parameters characterizations, such as the manufacturing origin of PV modules, could lead to significant modifications of the environmental performances. A new parameter is defined (e.g. “manufacturing scenario”) and characterized by two states: the current state (e.g. current manufacturing origin) and the future state (e.g. future manufacturing origin) with a given probability distribution between the different states. Global sensitivity analysis is used to determine whether this new parameter induces a significant part of the performances variance. A new simplified model is then constructed to assess the prospective impacts of the considered energy pathway.

39

Will organic photovoltaic technology render benefits in a 30-year horizon?

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Today, worldwide energy production is largely relying on the use of fossil fuels, which pose significant environmental problems and emphasize the need for an increasing utilization of renewable energy sources. By 2060, solar generators are foreseen to take up a major share of the world’s energy supply. From that scenario, organic photovoltaics (OPV) have recently emerged as promising solar technologies for large-scale energy supply systems. However, they will only have a key role if they are engineered to ensure sustainable and efficient power networks in the long term. Therefore, when applying life cycle assessment (LCA) to quantify their environmental impacts, a dynamic perspective is required. In this study, we aim to assess the life cycle environmental performances of an average annual supply of 6 PJ_{el} of electricity to the grid from an OPV-based solar park within the coming 30 years. Because of the intermittent nature of the energy supply, alternative sources are included in the modeled system for compensating the lack of solar electricity generated in days with no sufficient sunlight. We set up a large set of forecasting scenarios to account for temporal variations in (i) the efficiencies and lifetime OPV modules, (ii) the different average and marginal energy mixes used for both energy requirements and crediting, and (iii) the end-of-life scenarios, e.g. recycling efficiencies. Our results can demonstrate that a number of parameters have a large influence on the LCA results. For example, trade-offs and burden-shifting may occur between the increasing efficiencies of the OPV system and the decreasing impact savings from incineration with energy recovery as crediting energy mixes increasingly include more renewable sources over time. Based on a comprehensive overview of the key parameters that lead to such trade-offs and burden-shifting within the considered 30-year time horizon, we provide recommendations to technology designers and decision-makers for them to anticipate and tackle potential environmental impacts as early as possible in the development and deployment of the OPV technology for large-scale energy systems.

How can scientific advances support regulatory risk assessment for pesticides? (I)

40

Sloped mesocosms: a new design to evaluate macrophyte community effects

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Recent guidance on the conduct and interpretation of mesocosm tests highlights the importance of using the most appropriate aquatic communities for the chemical being investigated. In addition, it is equally important that for robust effects assessments, the selection of appropriate measurement endpoints and ecological evaluations is critical to the derivation of powerful statistical results. Here we will show the progress that has been made in this area of mesocosm study design using examples drawn from recent state of the art regulatory tests with herbicides. When conducting a mesocosm study it is essential that a sufficient number of representative macrophytes from sensitive taxonomic groups are included. We identified eight macrophyte species suitable for use in mesocosm testing that represent a range of functional groups and morphological traits such as rooted floating leaved monocotelydons and rooted submerged dicotelydons. In order to include sufficiently diverse taxa, marginal plant populations have traditionally be raised in the water column of deep mesocosms to facilitate their growth. However, this approach does not allow for true community effects to be determined. To rectify this, CEA have developed sloping mesocosms that enable macrophytes to be planted at water depths that most accurately reflect natural edge of field water-bodies allowing for true communtiy effects to be evaluated. Finally, for a robust assessment of macrophyte health we identified a need to collect quantitative as well as qualitative data. We did this by developing a range of assessment criteria based on macrophyte morphological characteristics and ecological functions e.g. percentage of chlorosis, stem length, and number of flower spikes, leaves or stems. in order to allow for true community effects to be evaluated Minimum Significant Difference classes for macrophyte assessments in this study were generally Class 3 or Class 4 with some occasional higher values. As a result, the endpoints for macrophytes were considered to be robust and reliable for evaluating the effects of a comprehensive and diverse macrophyte community. In addition to this; effects followed by recovery were also demonstrated for phytoplankton and zooplankton, indicating the use of sloped mescosms can allow for highly reliable and robust effects to be determined.

41

Addressing mesocosm data requirements in the new EFSA Aquatic Guidance Document

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The new EFSA Aquatic Guidance Document was published in August 2013 (EFSA, 2013). This document makes some new recommendations for designing and interpreting mesocosm studies. Changes include: the use of species sensitivity distributions (SSD) to identify organisms which should be present in the study based on lower tier work to ensure the establishment of a representative and sensitive aquatic community; the use of Minimum Detectable Difference (MDD) calculations to allow results based on low and variable data to be identified in order to avoid basing endpoints on results which are not statistically valid; new guidance for evaluating mesocosms studies and the identification and acceptability of threshold (ETO) or recovery (ERO) endpoints from these studies. A number of state of the art mesocosm studies have been conducted at CEA in which novel sampling and evaluation methods have been developed to produce data ranges with low MMD’s. Techniques have also been developed to improve the scope of organisms enumeration to provide the range of species data required in the new guidance.We will use these data to present reccomnedations for the acceptability of MDDs for different organisms groups e.g. zooplankton, algae and aquatic plants used in mesocom studies. We will also make further recommendations for the interpretation of these data and demonstrate how reliability indicies could be employed for the different groups evaluated in order to give anoverall assessment of the quality and reliability of mesocosm endpoints.

42

A proposal to address the minimum detectable difference (MDD) for the interpretation of treatment-related effects in micro-/mesocosm experiments

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In the European registration procedure for pesticides, microcosm and mesocosm studies are the highest experimental tier to assess the environmental effects of pesticides in edge-of-field surface waters. Evaluations of micro-/mesocosm studies are heavily relying on effect class concentrations based on NOECs calculated for different measurement endpoints. Ideally a power analysis should be reported to allow the identification of valid concentration-response relationships. The aim of this paper is to expand on the Aquatic Guidance Document published by the European Food Safety Authority [1] and to propose a procedure to report and evaluate the minimum detectable difference (MDD) in a harmonised way to facilitate the interpretation of micro-/mesocosm studies, with special reference to identify threshold concentrations of treatment-related effects and recovery potential of populations. Proposals for two decision schemes are presented that make use of MDD information to evaluate the reliability of a micro-/mesocosm study for the assessment of treatment-related effects of pesticide exposure on populations of water organisms and to derive effect classes that can be used in the derivation of Regulatory Acceptable Concentrations.

43

Assessing the environmental partitioning behaviour of pesticides: Polyparameter linear free energy relationship approach

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To assess potential risks is an essential step in the registration of pesticides. A key property for risk assessment is the partitioning behaviour of pesticides. Experimental measurements are often prone to large errors and require large efforts demanding time, costs, and resources. An alternative option is to predict equilibrium partition coefficients (*K*) using a model, such as a poly-parameter linear

free energy relationship (pp-LFER). The pp-LFER provides accurate predictions for various environmentally relevant systems. However, the necessary descriptors are often missing for pesticides. Hence, we determined pp-LFER substance descriptors for 86 pesticides and pesticide transformation products in this work as well as descriptors for 25 other environmentally relevant compounds. The complete set of descriptors for 50 compounds incorporating 47 pesticides was determined for the first time. The experimental determination is based on GC-retention times (log t_{net}), organic liquid/organic liquid and PDMS/water partition coefficients. The determined substance descriptors are internally consistent indicated by a root mean squared error (rmse) between experimental and pp-LFER predicted log *K*/ log t_{net} of 0.07 on average. The determined descriptors were validated by comparison of predicted and experimental literature partition coefficients for the systems octanol/water (K_{ow}), water/air (K_{wa}), and organic carbon/water (K_{oc}), resulting in a reasonable prediction accuracy, e.g., the rmse is 0.39 for log K_{ow}. This agreement can be regarded as high because our descriptor determination is independent of K_{ow} and the literature values originate from diverse sources. Comparing our descriptors to previously published ones reveals an improvement in accuracy in most cases, if published descriptors were available at all. For instance, the rmse for log K_{wa} using descriptors determined by Tülp et al. is 1.39 for the 26 compounds present in both data sets. Applying our descriptors results in an rmse of 0.89 log units. The presented descriptors substantially extend the descriptor availability toward the types of chemicals that are different from typical neutral organic chemicals in terms of solvation properties.

44

Histochemistry as a post-mortem diagnostic tool for organophosphate toxicosis in birds, implemented within a monitoring program in citrus orchards in Spain

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Cholinesterases are ubiquitous enzymes which are present in abundance in animals, in particular in the nervous system. The activity of cholinesterases, inclusive of both acetyl-cholinesterase and butyryl-cholinesterase, has been commonly used as a biomarker for exposure evaluation and risk assessment. Ten citrus orchards in Spain treated with chlorpyrifos were selected as sites for a 3-year monitoring to provide information on diversity and abundance of bird communities. Within this program, on one site, there were incidental observations of spray solution being spilled during sprayer-filling, and also spraying whilst puddles of water were present due to leaking irrigation pipes and overwatering. On this site, bird carcasses were discovered, mostly of blackbirdsand it was considered likely that the poor practises lead to the deaths. The carcasses were collected, stored at -20ºC and subsequently processed for post mortem examination and histochemistry in order to ascertain the cause of death. Following a strict protocol of post mortem, tissue samples (brain, liver, heart, duodenum and kidney) were collected and fixed in paraformaldehyde. Brains were sectioned in a cryotome and then processed for haematoxylin-eosin staining and acetyl- and butyryl-cholinesterase histochemistry using various substrates and inhibitors. Most birds appeared normal during post mortem, with no evidence of disease or parasitic infection; some cases of parasitic worm infections, which visibly interfered with the overall poor condition of the birds, were detected. These parasitic worms completely blocked the gastrointestinal tract of the infected birds, rendering them unable to pass food and thus resulting in low body and fat scores, possibly even to death. The histochemical analysis of the "normal in post mortem" carcasses revealed, for some birds, evident signs of organophosphate poisoning. The cyto-architecture of the brain was disrupted (haematoxylin-eosin staining) while esterase histochemistry confirmed toxicosis presumably by chlorpyrifos. It is concluded that the combination of post mortem and brain section haematoxylin-eosin staining and acetyl- and butyryl-cholinesterase histochemistry using various substrates and inhibitors, constitute a useful novel approach for determining organophosphorus-related toxicosis in birds tissues. These results lead to implementation of stewardship measures at the orchard in question. *Keywords: birds; chlorpyrifos; cholinesterase; histochemistry*.

45

Varying levels of protection in current risk assessment of birds and mammals: How to reach harmonisation

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In current risk assessment, practical approaches are preferred, sometimes based on rather simple calculations, which shall ensure that no unacceptable risk is posed on non-target organism. Since it is difficult to take account of all biological diversity (regarding the toxicity of different species, exposure or behavior) with simple approaches, safety factors are added. However, these safety factors were not always derived based on scientific evidence. Safety factors may be very protective in one species and less protective in another species. Based on examples for the risk assessment of birds and mammals, in the present study we show how the level of protection varies. The application of the same risk assessment approach for different species and application types results in different levels of protection. Especially, when using higher tier approaches, additional safety factors are sometimes proposed, although the overall uncertainty of the approach is reduced by including more scientific evidence. As a consequence, the level of protection of such approaches is often increased by additional safety factors. This is demonstrated based on different examples from standard risk assessment, refined risk assessment and mechanistic modeling studies. We show how the level of protection could be harmonized.

Plants and pollutants in the environment (I)

46

Impact of single and multiple pesticide pollution on various endpoints in Myriophyllum spicatum cultivated in sediment-water or culture medium only

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To reduce pesticide contamination of aquatic systems, buffer zones are recommended. In Lorraine, drainage ditches have been installed at the outflow of agricultural sites to reduce pesticides export. To evaluate risks emerging from pesticide run-off for aquatic systems, we exposed *Myriophyllum spicatum* to three pesticides (flufenacet/FA, isoproturon/IP, mesosulfuron-methyl/MSM) typically found in Lorraine. We tested how culture condition (sediment-water, medium, nutrient availability) affect common and new endpoints in this potential new OECD test system. Apical shoots of *M. spicatum* were cultured in sandy sediment and artificial freshwater. Pesticides were dosed at 100 μg/L in single exposure, and 3x33 μg/L or 3x100 μg/L to each 2 apical shoots per beaker, with sampling at days 0, 3, 7 and 21. We determined pesticide concentration in water, plant growth (length & biomass of shoots and roots), chlorophyll, anthocyanin and phenolic compounds. All herbicides were degraded but with different kinetics. Biomass did not increase much in all treatments due to nutrient limitation. Root biomass reacted much stronger than shoot biomass, and was significantly lower for all single and mixed pesticides treatments, but specifically in all treatments containing MSM, resulting in root:shoot ratios of 1.0-1.3 for control, IP, or FA, but only 0.2-0.3 for MSM and mixed treatments. These findings support prior notions that roots are a very sensitive endpoint for *Myriophyllum*, even when pesticides were applied via water. We strongly encourage to keep root biomass and root:shoot ratio as further endpoint in the sediment-water bioassay. IP caused sometimes higher chlorophyll, anthocyanin and TPC levels. The latter two are plausible considered the potential antioxidative action of phenolic compounds. The next series of experiments will include a sediment-water test based on enhanced nutrient availability, but also liquid-only tests with axenic cultures. We expect that culture conditions will affect the plant's response towards pollutants. The rather low effects observed here – with exception of the root response – might result from nutrient limitation. We finally aim to test the use of *M. spicatum* in situ in drainage ditches to monitor the effects of pesticide multi-contamination. The combination of various test systems should allow us to evaluate the best *M. spicatum* test system for pesticide run-off in agricultural areas.

47

Myriophyllum spicatum's polyphenols as a new sensitive and informative parameter for pollutant impact assessment on aquatic ecosystems

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The quest for environmental relevance is shaping contemporary ecotoxicology. It is no longer sufficient just to measure the effects of a pollutant on a given organism, but rather to assess the effects of environmental pollution on community interactions and ecosystem functions. Ecosystem level consequences can only be extrapolated from simplified systems, such as micro- or mesocosm studies or field experiments. These tests, however, are complicated to set up. We propose another possibility: to study species known for their biotic interactions, and see if a disruption of these interactions will induce functional changes at the community or ecosystem level. In this context, *Myriophyllum spicatum* proposed by the OECD as future test organism is a good candidate. This plant produces large amounts of polyphenols involved in multiple responses towards abiotic and biotic stressors, i.e. they are effective allelochemicals. The pro- or antioxidant properties of these polyphenols also make them a parameter which may vary in response to environmental stressors such as pollutants. We thus propose to analyse these molecules, in addition to endpoints commonly used in ecotoxicology such as growth and pigment content. We thus performed two experiments with axenic cultures of *M. spicatum* in liquid medium, using factorial designs, to investigate a) the effect of cadmium (Cd) and sucrose-addition to the medium, and b) the impact of arsenic (As) depending on the nitrogen to phosphorus molar ratio (N:P) of the medium. The results show that (i) both Cd and As as well as the composition of the medium can significantly affect the metabolism of *M. spicatum*, and that (ii) depending on the parameter considered, the composition of the medium modified the effects of the pollutant. Polyphenols increased under all conditions with Cd exposure, and with As but only under high N:P ratios. Modifications of these allelochemicals will likely affect the impact of Eurasian watermilfoil on other organisms. Bioassays with algal competitors and herbivores are currently being developed. Our first results support our initial hypothesis that *M. spicatum* is a good candidate for risk assessment at the ecosystem level because of the indirect effects of pollutants on allelochemical interactions. The variable responses in result of the variations of the culture medium may help to understand variable responses found in situ, where conditions such as resource availability, also vary.

48

Simplifying the application of a Myriophyllum spicatum TK/TD growth model by estimating chemical specific, toxicokinetic parameters

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Toxicokinetics of chemicals are a key component for evaluating the effects of time variable exposure, especially in aquatic systems, where organisms are regularly exposed to pulsed concentrations of chemicals, such as plant protection products. Mechanistic, toxicokinetic and toxicodynamic models offer a promising approach to increase the knowledge of effects caused by time variable exposure by extrapolating constant exposure profiles to more complex ones. Instead of considering concentrations in the water phase, outside organisms, TK/TD models can link effects to concentrations within organisms, thereby increasing the realism in the effect assessment of chemicals. This work shows how toxicokinetic parameters, necessary to use a previously developed TK/TD growth model of *Myriophyllum spicatum*, can be estimated for chemicals without conducting additional experiments by using physicochemical properties of the respective chemicals. The parameterized TK/TD growth model of *M. spicatum* can be used to predict uptake and elimination patterns of chemicals, as well as their distribution between plant compartments. By using the model, we show that the lag in the onset and the disappearance of effects can be explained by toxicokinetics, at least for a sufonyl-urea compound. A general approach is presented how complex exposure profiles of chemicals might be evaluated, in respect to the growth of *M. spicatum*, without conducting additional experiments considering individual exposure profiles.

49

The use of Ceramium tenuicorne growth inhibition test for testing chemicals, products, effluent waters as well as contaminated soil and sediments

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The marine red macroalga *Ceramium tenuicorne* is cosmopolitan and naturally found in temperate waters in both brackish and marine environment. A growth inhibition test has been developed based on two clones, one originating from 7 ‰ and the other from 20 ‰. This test became an ISO standard in 2010 (ISO 107 10) for testing of chemicals and water effluents. The test has now been further developed for soil and sediment samples. This test has been used for testing of single chemicals exhibiting high sensitivity, e.g. EC50 of 20 – 33 μg Zn^{2+}/L in 7‰ and 32-61 μg Zn^{2+}/L in 20 ‰ and for copper 1.9-3.8 μg Cu^{2+}/L in 7 ‰ and 7.9-13 μg Cu^{2+}/L in 20 ‰. The species is highly sensitive to effluent waters from pulp mills. The Ceramium growth inhibition test was used in a test battery together with a life cycle test with the crustacean *Nitocra spinipes* and a 2-generation test with the zebrafish. The alga was the most sensitive of these species to all 14 tested effluent waters. Leachates was prepared from different anti-fouling paints and used for ranking different paints according to their toxicity to non-target organisms. The alga was generally more sensitive to the compounds leaking from the paints than the crustacean *N. spinipes*. Sediments from small town harbours and from natural harbours used for anchoring by pleasure boats, was collected and leachates were prepared and tested with the growth inhibition method with *C. tenuicorne*. The result show that this method could rank the harbours according to their toxicity and proved the small town harbours to be most toxic and that compounds originating from antifouling paints was responsible for a large part of the inhibiting effect. Soil from boatyards was used for preparing lecahates, which was tested with the bacterium *Vibrio fisherii* in the Microtox test, growth inhibition of the macrophyte *Myriophyllum aquaticum* and the alga *C. tenuicorne* and larval development rate of *N. spinipes*. The alga proved to be the most sensitive species to the contaminants found at such places. *C. tenuicorne* has been exposed to many different types of pollutants both single chemicals and mixtures in effluent waters, sediments and soils and shown high sensitivity to all tested applications. Because of the sensitivity of the alga, its relevance for large areas, the high reproducibility of the test, the ease of performing the test and the cost efficiency this test may be useful in a number of test batteries.

50

Accumulation of lead, zinc and copper in different organs of Typha domingensis grown in an abandoned mining area

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Studies based on the absorption and accumulation of heavy metals in macrophytes as *Phragmites australis* or *Bolboschoenus maritimus* are increasing due to its use as a low-cost and ecological alternative to decontaminate effluents and sediments affected by this kind of pollutants. The aim of this study is to know the ability of *Typha domingensis* to accumulate Pb, Zn and Cu in different organs under field conditions, and its possible application as phytoextraction technology in mining regions. The study area was located along the Valdeazogues River which flows through the Almadén mining district, where among the mercury extraction, other important mining activities where performed to obtain metals such as Pb, Zn, Cu and Fe. In this way, samples of *T. domingensis* and sediments under the plant samples were collected along the river basin to know their contents in heavy metals. The samples of Typha were divided in four fractions: leaves, rhizomes, root and root cores (the joining point of rhizome, roots and leaves). The results obtained for each studied heavy metal, showed that roots and root cores were the organs with the highest accumulations, followed by rhizomes, and ultimately, by leaves. Furthermore, the concentrations of metals in all tissues were several times higher than the concentrations of available metal in the environment, reaching, in the case of lead in roots, up to 6 times higher than the surrounding sediment, and up to 180 and 23 times higher for zinc and copper, respectively. Therefore, these results have demonstrated that *Typha domingensis* can be a good candidate to be used in phytoextraction technology under real conditions in semiarid areas affected by lead, zinc and copper.

51

Uptake kinetics of inorganic and methyl mercury by two representative aquatic primary producers

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Understanding uptake and fate of metals in organisms is essential to predict the risk they represent in the environment.Mercury (Hg) is a toxic element which can be found as methylmercury (MeHg) and as inorganic form (IHg) in aquatic ecosystems. *Elodea nuttallii* is a submerged plant rooted in sediments, which has often been described as a tolerant species to metal contamination, with a high ability of bioaccumulation [1]. *Chlamydomonas reihnardtii* is a model microalgal species commonly used in ecotoxicity assessment. Both are representative of primary producers (macrophytes and phytoplankton) controlling Hg bioaccumulation and biomagnification in the food web in freshwater systems [2]. The aims of this study is to: (i) Assess if MeHg and IHg follow a similar uptake kinetics, and compare it to Cu. (ii) Localize subcellularly bioaccumulated metal, notably in cell wall and cell sap. (iii) Compare metal accumulation kinetics in two organisms that differ in their habitat, morphology, and tolerance, hence in their exposure. Exposure experiments were realised in the laboratory at 10^{-9} M IHg, 10^{-10} M MeHg or 10^{-6} M Cu during 10, 20, 30, 45 min, 1, 2, 4, 6 or 8 hrs. This study highlights the fact that in *Elodea nuttallii* IHg and MeHg may bioaccumulate by different pathways or be reagulated by different mechanisms, because their kinetics, as well as their subcellular distribution are different. We will then compare this result with Cu and with bioaccumulation kinetics in *Chlamydomonas reinhardtii*. References: [1] Regier N *et al*.. 2013. Mercury bioaccumulation in the aquatic plant *Elodea nuttallii* in the field and in microcosm: Accumulation in shoots from the water might involve copper transporters. Chemosphere 90(2): 595-602. [2] Bravo AG *et al*.. Extremely elevated methyl mercury levels in water, sediment and organisms in a Romanian reservoir affected by release of mercury from a chlor-alkali plant. Wat. res.(in press).

REACH after the second registration deadline: Environmental challenges (I)

52

REACH related environmental information: status and prospects for its availability and quality

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The European Commission review of the REACH Regulation in 2013 indicated a marked increase in the quality of data as a result of the first REACH registration phase as well as a marked decrease in the risk associated to substances already registered. For example, increased information is resulting in changes in hazard classification, with the majority becoming more stringent. However, the Commission review also indicated that many registration dossiers have been found to be non-compliant with regard to their information, as well as containing insufficient assessments by registrants of persistent, bioaccumulative and toxic (PBT) and very persistent, and very bioaccumulative (vPvB) properties. Improved

quality of the information in the REACH registration dossiers is one of the strategic objectives of ECHA to enable the safe manufacture and use of chemicals. High quality information is scientifically sound, understandable and reliable. In ECHA's view industry needs to take full ownership of its registration dossiers and proactively work on their quality, even after submission to ECHA. ECHA has strengthened and continues to enhance its dossier compliance check activities and other measures to improve the dossier quality. ECHA has by the end of 2013 concluded compliance checks for over 1 000 registration dossiers over 100 tonnes submitted for the first REACH registration deadline. 69% of these evaluated dossiers were found to be non-compliant. ECHA is focussing it compliance checks on the dossiers of highest concern with initial focus on the endpoints of highest relevance for safe use, later address exposure and chemical safety assessment. Second related ECHA's strategic aim is to mobilise EU authorities to use the REACH registration data intelligently to identify and address chemicals of concern. The REACH Registration Database maintained by ECHA contains currently information on 12 276 unique substances from 47 097 registration dossiers (including ones from previous legislation). The information is a valuable resource for advancing the safe use of chemicals and for the replacement of the most hazardous ones by safer alternatives. The latest new type of information made available is whether a chemical safety assessment was performed and the results of the PBT and vPvB assessments. ECHA plans next to improve the way the information is presented in the search results: it will be short substance summaries. Information on chemical and (eco)toxicological properties of REACH registered substances per endpoint is accessible via eChemPortal.

53

How toxic is an industrial chemical? A distribution-based assessment of hazard estimates from the REACH registration data

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54

Challenges and Solutions in Environmental Risk Assessment for Petroleum Substances under REACH

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55

Strengths and weaknesses of implementation of bioavailability correction tools for metals

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The bioavailability of metals in different environmental compartments depends on the physico-chemical properties of the environmental media and on competition for binding at the biological membrane level. Bioavailability explains to a great extend the variation in toxicity response of standard toxicity tests using media with different physico-chemical properties and the often observed discrepancy in metal toxicity between laboratory and field conditions. Normalizing these responses using bioavailability correction models is therefore a crucial step in the determination of environmentally safe levels for the various environmental compartments and should hence be applied in metals hazard and risk assessments, for example under REACH. Such models have been developed for several representative biological species for the aquatic ecosystem (Biotic Ligand Models), for the sediment compartment (AVS-SEM correction model) and more empirically based regression models for sediment/soil organisms. While the application of such models will reduce the variability caused by varying abiotic conditions, their application for regional or continental scale environmental risk assessments like used under REACH, can be challenging. Aspects like, the selection of bioavailability models, the representativity and applicability of the models available for the overall species biodiversity, the spatial and temporally variability of abiotic factors, as well as co-exposure to other metals raise the need for strategic concepts and uncertainty management to define the conditions under which they can be applied in a standard way for chemicals management. This presentation aims for demonstrating such experience with case studies for a series of metals for the aquatic, sediment and soil compartments.

56

Experiences from regulatory risk assessors on Environmental Hazard and Risk Assessment

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Experience gained in the past few decades on hazard and risk assessment of chemical substances for regulatory purposes is usually documented in guidance documents (GD's). To fill some data gaps for more complex substances and topics that are not covered by available guidance documents, conceptual models have been specifically developed. For example, models based on hazardous properties and chemical fate have been developed and used to prioritize substances for regulatory purposes and for risk assessment. Both regulators and industry apply such models. RIVM has developed the PB score, a model that ranks substances along a persistence and a bioaccumulation scale. Industry applies specific models for the risk assessment of difficult substances such as complex mixtures and metals. Examples of these are the PETROTOX model and the target lipid model for petroleum hydrocarbons and the biotic ligand models applied to several metals. \n The use of such models for hazard and risk assessment should comply with the general requirements of Annex XI of REACH, namely that i) they should be adequately and reliably documented ii) the scientific validity of the model should be established in a validation study \n and iii) they should be be adequate for the purpose of risk assessment and classification and labelling. The remaining uncertainty in a modelling exercise should be taken into account. Each modelling approach will bear some inherent uncertainty that should be adequately addressed. This can be done according to various degrees of refinement, ranging from the use of pragmatic assessment factors or taking statistical and inherent prediction of uncertainty into account. This presentation will focus on principles of model application and will identify success factors as well as limitations for regulatory risk assessment.

57

Discussion

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Ecotoxicology in tropical and polar regions (II)

58

DDT Dynamics in a Tropical Floodplain Lake

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Monitoring and modeling of persistent organic pollutants (POPs) have mostly centered in temperate and cold regions, neglecting tropical and dry subtropical zones, which account for nearly half of the earth's surface and are critical for the support of biodiversity. DDT is an organochlorine POP, whose use is currently restricted to indoor residual spraying (IRS) for malaria vector control. DDT use in IRS in Brazil was reduced in the mid-90s and definitively banned by federal law in 2009. However, studies in an Amazonian settlement around Lake Puruzinho have found elevated DDT concentrations in fish and breast milk, which result in an infant daily intake four times higher than the World Health Organization standard. To describe the sequence from DDT emissions in the Puruzinho environment to human exposure, a dynamic multimedia mass balance environmental model was coupled to simple steady-state fish bioaccumulation and human pharmacokinetic models using the fugacity approach. The temporally resolved environmental model effectively captured the seasonality of the hydrological cycle and floodplain dynamics, which influence the distribution of DDT in the Puruzinho environment. DDT concentrations in the different environmental compartments followed a yearly seasonal pattern, with elevated water and sediment concentrations, but low soil and air concentrations during the high water season. As expected from its high octanol-water partitioning coefficient, DDT accumulated in the soil and sediments. The main processes driving intermedia DDT fluxes were soil runoff, deposition of suspended particles in the water column, and gaseous and wet deposition from the air. Water and sediment concentrations from the environmental model were used to calculate DDT concentrations in fish, which in turn were used as to calculate the DDT human body burden, as fish is the main source of protein for the Puruzinho inhabitants. Lipid normalized concentrations in humans based on the model results revealed that fish intake accounts for less that 10% of the DDT found in breastmilk in Puruzinho. By developing the first multimedia environmental model that describes POP dynamics under the highly variable environmental conditions that characterize tropical floodplain lakes, we have made an important step towards filling in significant knowledge gaps about the fate and transport of POPs in tropical ecosystems.

59

Biota-sediment accumulation factors and trophic magnification factors: evaluation of the applicability for subtropical and tropical aquatic systems and a comparative study between the two regions

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The fate and trophic transfer of persistent organic pollutants (POPs) in aquatic ecosystems are intensively investigated in temperate regions, but for (sub)tropical regions a large data gap still exists. Subsequently, risk assessments in (sub)tropical countries often rely on temperate toxicity data, although it may be debatable whether the fate of chemicals is comparable among geographically distinct ecosystems. Two risk assessment tools which are used in temperate aquatic ecosystems to investigate bioavailability, bioaccumulation and bio-magnification of POPs are (1) the biota-sediment accumulation factor (BSAF) and (2) trophic magnification factors (TMFs). The present study aims to evaluate the applicability of these two tools for subtropical and tropical aquatic systems. In addition, a comparative study of the TMFs in those two regions is presented. Samples of sediment and biota (i.e. invertebrates and fish species) were collected in a tropical (Congo River, DR Congo) and a subtropical river (Olifants River, South Africa). POPs (PCBs, PBDEs, DDTs, HCHs, CHLs and HCB) were determined by GC-MS. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) measurements were performed using an EA-IRMS. For both rivers, BSAFs were higher than BSAFs found in temperate regions, probably caused by different mechanisms. POP levels in sediment are a poor indicator of the real exposure and bioavailability in these environments, because different dissipation processes (volatilization, atmospheric dispersal and faster rates of degradation) may play a significant role in (sub)tropical areas. For all POPs, no or inverse relationships were found between the sediment concentrations and BSAF values. This implies that, in the conditions of the present study, the BSAF concept appears to be a poor predictor of the bioavailability of POPs in subtropical and tropical rivers. In both rivers, significant positive relationships between relative trophic level and lipid-normalized POP concentrations were observed so trophic levels play an important role in the movement of contaminants through the food web. TMFs were > 1, indicating biomagnification of POPs. For PCBs, no differences in TMFs were found between the two rivers. For p,p'-DDT, a higher TMF value was observed for the subtropical Olifants River (5.2) than for the tropical Congo river (1.7). This observation can be caused by a higher degree of transformation of p,p'-DDT to the corresponding metabolites in the Congo River compared to the Olifants River.

60

Distribution, mobility, and pollution assessment of Cd, Cu, Ni, Pb, and Fe in intertidal surface sediments of Sungai Puloh mangrove area, Malaysia.

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Sungai Puloh mangrove supports a great diversity of macro benthic organisms and provides social benefits to the local community. Recently, it has become a major recipient of heavy metals as a result of industrialization and urbanization. This study was conducted to evaluate mobility and pollution status of heavy metals (Cd, Cu, Ni, Pb, Zn, and Fe) in intertidal surface sediments of this area. Surface sediment samples were collected based on four different anthropogenic sources: (I) effluents from recycling factory, (II) urban runoffs and effluents from coal-fired power plant, (III) fishermen activities, and (IV) Shipping activities. The heavy metals concentrations were analyzed using an atomic absorption spectrophotometer (AAS). Results revealed that the mean concentrations were Zn (1,023.68±762.93 μg/g), Pb (78.8±49.61 μg/g), Cu (46.89±43.79 μg/g), Ni (35.54±10.75 μg/g), Cd (0.94±0.29 μg/g), and Fe (7.14±0.94%). Several quality guidelines and environmental indices were used to evaluate the levels of heavy metals concentrations. Most of the mean values of analyzed metals were below both the Interim Sediment Quality Guidelines (ISQG-low and ISQG-high) except for Pb concentration (above ISQG-low), and Zn concentration (above ISQG-high), thus suggesting Pb and Zn may pose some environmental concern in this study area. Cadmium, Pb, and Zn concentrations were above the threshold effect level (TEL), indicating seldom adverse effect of these metals on macro benthic organisms. Pollution Load Index (PLI) indicated deterioration, while other contamination indices showed that the intertidal surface sediment is moderately polluted with Cd, Pb, and Zn. Therefore, the mangrove area requires urgent attention to mitigate further contamination. Finally, this study being a base line study with detailed contamination assessment indices, will contribute to data sources for Malaysia in establishing her own Interim Sediment Quality Guidelines.

61

Aluminium toxicity in tropical montane forest soils: Response of nutrient uptake to elevated Al concentrations

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An undesirable feature of acid tropical soils (pH < 5.5) is high aluminium mobility in soil solution, which, depending on plant sensitivity, may cause phytotoxicity. In southern Ecuador, Amazonian forest fires lead to increased H and N deposition, which cause acidification of the ecosystem and could result in increased Al mobility and thus, increase the risk of phytotoxicity to Al-sensitive plants. To investigate the impact of Al on selected tree species in southern Ecuador, an hydroponic experiment was conducted. Seedlings of *Cedrela odorata* L., *Heliocarpus americanus* L., and *Tabebuia chrysantha* (Jacq.) G. Nicholson were treated with an Hoagland nutrient solution containing 0, 300, 600, 1200, and 2400 μM Al. Additionally, we grew seedlings of the same tree species in native litter leachate. Nutrient solutions were sampled and replaced weekly. After seven weeks, roots, stem, and leaves were separated and digested. Macro and micro nutrient concentrations in nutrient solutions before and after treatment and in plant tissue were determined with AAS, ICP-MS, and TOC and CNS analyzers. After treatment, concentrations of remaining macro and micro elements and TOC in nutrient solution at the end of the experiment increased with Al concentration illustrating decreasing plant uptake. Concentrations of Mg and Ca in plant tissue decreased with increasing Al concentration and those of P, Fe, and Ni showed first enhancement at 300 μM Al and then decreased at higher Al concentrations. Aluminium stress affected nutrient uptake negatively and thus reduced biomass production of tree seedlings. Yet, Al toxicity occurred at Al concentrations between 300 and 600 μM, which is far above usual total Al concentrations (< 60 μM) in native litter leachate, the plant-available Al pool. We conclude that at the present concentration levels of Al in litter leachate, Al toxicity to the investigated tropical montane tree species in southern Ecuador is unlikely.

62

Organic pollutant effects on the photosynthetic gene expression of natural communities of Prochlorococcus sp.

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Semivolatile persistent organic pollutants are introduced into remote oceanic regions by atmospheric transport and deposition, with the subsequent effects on aquatic organisms. Previous studies proved that organic pollutants present in seawater affect abundance, growth rate and cell viability of phytoplankton, suggesting that mixtures of contaminants could influence ecosystem functions like oceanic CO_2 fixation or productivity. The cyanobacterium *Prochlorococcus* numerically dominates the photosynthetic community in the tropical and subtropical regions of the world's oceans, so its contribution to primary production is significant. Being the smallest known photosynthetic organism, its tiny size and spherical shape provide a high surface to volume ratio, making it very prone to acumulate organic chemicals and particularly sensitive to pollutants. . As a part of a large project to evaluate the effects of organic pollutants on the marine carbon cycle, the potential impact of organic pollutants on photosynthetic function of phytoplankton was performed during the Malaspina circumnavigation cruise (December 2010 - July 2011). A total of 16 experiments were performed in the Indian, Pacific and Atlantic Oceans. Natural communities from the deep chlorophyll maximum (DCM) depth were challenged with three different organic pollutant mixtures (PAHs; HCB+HCHs; and a complex mixture form concentrated seawater) and incubated at 4 exposure times. To monitor the effects of the treatments on the photosynthetic function, mRNA abundances of *rbc*L (RuBisCO), *psb*A (D1 protein) and *rnp*B (reference gene) for each of the two major *Prochlorococcus* clades (high light and low light), were assessed by qRT-PCR for each experiment. Preliminary results show that the complex mixture of pollutants produced a decrease of *rbc*L expression after 6h and 24h of exposure, particularly for the high light clade, whereas the other two mixtures tested did not induced any effect. This confirms an effect of cocktails of pollutants at environmentally relevant concentrations that it not observed when we tested single families of them at similar concentrations. The downregulation was only found in *rbc*L while *psb*A remained unaltered, which suggests a specific effect on the CO_2 fixation but no on the complete photosynthetic apparatus. The decrease of *rbc*L expression together with effects at cellular level could exert a perturbation on the marine carbon cycle.

Marine and coastal ecotoxicology and risk assessment (II)

63

The toxicity of chemical warfare agent mixtures on the Baltic mussel Mytilus trossulus

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After the World War II, approx. 40.000 tonnes of chemical munitions containing about 13.000 tonnes of chemical warfare agents (CWAs) were dumped in the Baltic Sea. As the artillery shells, aircraft bombs and containers are corroding, the contents are leaking into the environment contaminating the surrounding sediments and beyond dumpsite boundaries. Thus, the necessity of investigations on the impact of chemical warfare on biota is increasing. Here, we present the first approach to investigate biological effects of CWA mixtures on the health of blue mussels (*M. trossulus*). The evaluation of the health status was based on a wide array of biomarkers at various biological levels ranging from molecular to cellular

and functional endpoints; encompassing immunocompetence, oxidative stress defence and pathological alterations in different tissues. Chemical analysis of tissue and water samples facilitated an integrated assessment. Individuals of the Baltic blue mussel (*M. trossulus*), collected at a pristine site in Åland (SW Finland, 5.4 ‰ S, 8°C), were randomly distributed and exposed to 13 differing mixtures of the CWAs Clark I, Adamsite and Chloroacetophenone at environmentally relevant concentrations (from 1.25 μg/L to 50 μg/L), solvent controls (50 μl/L methanol) and controls (no added chemicals) for 96 hours. Each treatment consisted of 8 glass aquaria (12 L) containing 7 mussels. Water change (artificial seawater: Coral Pro Salt, Red Sea) and re-dosing was conducted daily. Preliminary results suggest a differing impact of CWA mixtures on biochemical and functional endpoints. Whilst, the antioxidant glutathione reductase activity was affected by the arsenic compounds Clark I and Adamsite, all three CWAs had a stimulating impact on phagocytic activity based on the respective multiple linear regression model. Thus, reflecting differing modes of action of the CWA mixtures. Further data assessment of the above-mentioned toxicity endpoints is still ongoing, which will be presented at the conference. Results obtained in the present study, combined with results of a previous mussel transplantation study as well as spatial information of dumped CWAs, shall support and enable the establishment of CWA leakage models in the Baltic Sea and improve the risk assessment models. The present study was conducted within the framework of the CHEMSEA project, which was part-funded by the European Union (Baltic Sea Region Programme 2007-2013)

64

Effects of persistent organic pollutants on marine primary production: a modelling approach

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Due to their ability to accumulate in fatty tissues, persistent organic pollutants (POPs) are an issue of environmental concern. The presence of POPs in the marine environment may be a potential driver impacting primary production [1], but their the role as a growth-limiting factor has rarely been explored [2]. In this study we assessed to what extent POPs impact the phytoplankton dynamics in the southern part of the North Sea. Our approach consisted of fitting a nutrient-phytoplankton-zooplankton (NPZ) model to *in situ* data and testing whether the extension of this model with POP toxicity improved the model fit. The NPZ model was run for four different model configurations, one model configuration contained no POP toxicity and three configurations included POP toxicity, being (1) the ambient POP concentration (1TOX); (2) a tenfold of the ambient POP concentration (10TOX); and (3) a hundredfold of the ambient POP concentration (100TOX). All model configurations predicted the chlorophyll a concentrations reasonably well, as most observed data were situated between the 2.5% - 97.5% confidence interval of the NPZ simulations. The inclusion of organic pollutants did not improve predictions. When the POP concentrations were multiplied by 10 (10TOX) and 100 (100 TOX), the median model deviation remained unchanged, but the variance of the model deviation decreased. This can be explained by the fact that at 10TOX and 100TOX, the POP toxicity forcing function in the NPZ-model became relatively important compared to the dynamics of the conventional phytoplankton drivers such as solar radiation, nutrients and water temperature. Based on the methodology presented in this paper, the relative importance of POP toxicity on marine primary production was assesed. Along the Belgian coast, it was found that accounting for POP toxicity did not improve predictions of chlorophyll a dynamics, but reduced the variability of these predictions. [1] Halpern BS, Walbridge S,et al. 2008. A global map of human impact on marine ecosystems. Science 319: 948-952. [2] Echeveste P, Agusti S, Tovar-Sanchez A. 2012. Toxic thresholds of cadmium and lead to oceanic phytoplankton: Cell size and ocean basin-dependent effects. Environ Toxicol Chem 31: 1887-1894.

65

Biomarkers of oxidative stress as indicators of reproductive effects in the benthic amphipod Monoporeia affinis

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Multiple natural and anthropogenic stressors can cause shifts in the oxidative balance of cells. A shift in the balance, either through inducing the production of reactive oxygen species or depletion of the antioxidant defense, can cause damage to biomolecules, such as lipids, proteins and DNA. Changes in antioxidative capacity and oxidative damages are used in ecotoxicological studies as biomarkers of exposure to stressors, such as xenobiotics. Alterations in the antioxidant defense and increased levels of oxidative damages have been connected with organismal fitness and health. However, there are few studies investigating the potential of oxidative biomarkers as indicators of adverse effects at individual or population levels in ecotoxicological studies. Previous studies have shown that oxidative biomarkers in the benthic amphipod *Monoporeia affinis* are affected in response to xenobiotic exposure and hypoxia. In this study we investigated the relationship between alterations in biomarkers associated with oxidative stress, such as antioxidative capacity and lipid peroxidation, and embryo developmental disorders in *M. affinis*. Amphipods in this study were collected from the Baltic Sea, taken in to the lab and exposed to contaminated sediments for 14 weeks. Reproductive success was assessed at the end of the exposure by identifying and quantifying different types of embryo developmental disorders. Alterations in oxidative biomarkers in these organisms could provide a better mechanistic understanding of the observed reproductive effects. Moreover, by establishing a connection between biomarker response and effects at individual and population level, these biomarkers could be used as early indicators of later effects. This is important if we are to increase the value and use of oxidative biomarkers as effect biomarkers in environmental risk assessments.

66

Population growth rate in low- and high density populations

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Population growth rate is generally thought of as the key intervening variable linking individual level effects to effects on populations (e.g. 1). It integrates effects on survival, development and reproduction, and is therefore by definition a better ecotoxicological endpoint for population responses than any single individual level endpoint (2). The primary aim of the current study was to apply an equation for calculating population growth rate from a life cycle test where *Nitocra spinipes* were exposed to lindane: 0-29 μgL^{-1} (modified from (3)). Effects from stressors at the population level may be severely underestimated when population density is low (4). A secondary aim was therefore to compare population growth rate from a population test (high population density) and a life cycle test (low population density). In the population test, *N. spinipes* were exposed to lindane (in replicates of three): 0-28 μgL^{-1}. Populations were started with three memebers of from each development stage. Population growth rates were calculated and compared, and EC_{10} were calculated. Population growth rates from the life cycle test show that all populations were growing, and all lindane treatments were significantly different from the solvent control. In the population test, population growth rates for the solvent control and the lowest lindane exposure showed growing populations, the intermediate concentration was at the replacement rate for the population. The highest treatment showed a declining population, and was also significantly different to the solvent control. The few significant differences in the population test were a result of high variation. EC_{10} values (22.9 μgL^{-1} in the life cycle test and 11.9 μgL^{-1} in the population test), nevertheless, the population growth rates showed that the population test had stronger effects compared to the life cycle test. If NOEC/LOEC values were to be used, the life cycle test would have resulted in lower NOEC values compared to the population test. This is due to much higher statistical power (i.e. higher number of replicates and thus less variability) and does not reflect the actual effects seen on the population level. References: (1)Calow P, Sibly RM, Forbes VE. 1997. Environ Toxicol Chem 16:1983-1989. (2)Forbes VE, Callow P. 1999. Environ Toxocol Chem 18:1544-1556. (3)OECD 2009. Harpacticoid Copepod Development and Reproduction Test. (4)Sibly RM. 1999. Ecol Appl 9:496-503.

67

Effects of triphenylborane pyridine, tralopyril and capsaicin to marine invertebrates: do novel biocides still pose an environmental risk?

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Biofouling results from the settlement of organisms on water-submerged structures causing deterioration of diverse aquatic equipment and raising the fuel consumption by ships due to frictional drag. Antifouling (AF) paints have been used to overcome this problem and throughout history a variety of AF-biocides have been used to boost the paint efficacy. By the turn of the century tributyltin (TBT) was a key biocide in AF paints but its release to the environment resulted in well documented impacts to wildlife, leading to a global ban of TBT-based paints in 2008. Nowadays, the risk assessment of biocides assumes a critical importance for their commercial success. According to the new Biocidal Product Regulation (BPR, Regulation EU No 528/2012), implemented in the European Union in September of 2013, both the active biocidal substance and the commercial formulation have to be approved. This situation is prompting the industry to search for environmentally acceptable alternatives. A good AF performance, rapid transformation to less toxic products, low tendency to bioaccumulate and low toxicity to non-target species are required. The current work investigates the acute toxicity to marine invertebrates of two emergent AF biocides triphenylborane pyridine (TPBP) and tralopyril and a AF biocide candidate, capsaicin. The tested biocides impaired larval development in the mussel *Mytilus galloprovincialis*, inhibited larval growth in the sea urchin *Paracentrotus lividus* and caused mortality to the copepod *Tisbe battagliai* in a dose-dependent manner. Comparisons with TBT were made and the toxicity ranks obtained were TBT = TPBP > tralopyril >> capsaicin for bivalves, TBT > tralopyril > TPBP >> capsaicin for echinoids and tralopyril > TBT ~ TPBP >> capsaicin for copepods. Based on a marine antifoulant model to predict environment concentrations (MAMPEC, Version 3.0; Deltares, Netherlands) and on predicted no effect concentration, TPBP and tralopyril have the potential to harm the environment. Nevertheless, acute toxicity tests are just one of many steps towards the assessment of the environmental risk and therefore further research is essential to better understand their fate and effects.

68

Biomarker responses dependant of pollution source: effects of crude oil contamination through nutrition and water – a laboratory experiment with Baltic Sea blue mussel (Mytilus edulis)

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Oil-derived polycyclic aromatic hydrocarbons (PAH) induce many biomarker responses in organisms. These responses can lead to ecologically relevant effects at higher levels of biological organisation even at low toxin concentrations. In the case of oil-derived PAH bioaccumulation, the nutritional status of mussels can be especially critical since oil and its components can be digested as nutrient by phytoplankton that bivalves use as food A set of different biomarker tests was applied to assess the impacts of crude oil pollution from different sources on Baltic Sea blue mussel (*Mytilus edulis*). An aquarium exposure experiment of 25 days was conducted using water-accumulated fraction (WAF) of Russian crude oil in 1) aquarium water (1:500) or 2) feeding mussels with algae exposed to 1:500 WAF in growth medium. In two reference aquariums mussels were fed with clean algae. The mussels were fed with *Rhodomonas* sp. with final concentrations of either 8000 cells ml^{-1} or 1500 cells ml^{-1}. The algae were grown in T2-media in polluted or in unpolluted conditions, e.g. polluted *Rhodomonas* was grown in T2-medium contaminated with 1:500 WAF of Russian crude oil. The other four aquariums were fed with uncontaminated algae and two of the aquariums were exposed to water contaminated with 1:500 WAF of Russian crude oil. The biomarkers measured included condition index (CI), comet assay (CA), lipid peroxidation (LPO), superoxide dismutase (SOD), protein carbonylation, neutral red retention test (NRR) and lysosomal membrane stability (LMS). The crude oil was deployed as WAF with a concentration of 1:500 (oil/water). The stock solutions as well as mussel tissues were chemically analyzed for confirmation of the pollutant concentrations. Significant changes in comet assay and lysosomal stability occurred within 25 days of the beginning of the experiment, where lysosomal stability measured from histopathological samples produced more reliable results than NRR measured from blood haemocytes. Condition indexes were not correlated with the observed changes in PAH concentration, but in the availability of food. Oxidative stress biomarkers LPO, SOD, TAC and protein carbonylation showed less clear responses on oil pollution from nutrition or water but some responses to differences on nutritional status.

Waste and Wastewater effluents: chemical and ecotoxicological characterisation (II)

69

Toxicity and estrogenicity of aqueous bisphenol A samples treated by advanced oxidation processes

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Bisphenol A (BPA) is an organic pollutant with estrogen-like effects eliciting adverse effects on endocrine systems of humans and wildlife. For this reason an efficient removal of BPA from wastewaters, before discharging them into aquatic environment, is necessary. Several possibilities for endocrine-disrupting chemicals removal from water are available and among them advanced oxidation processes (AOPs), such as catalytic wet air oxidation (CWAO), heterogeneous photocatalysis, ozone-based technologies and ultrasound oxidation seem to be the most promising. However, treatment with these processes sometimes results in by-products that are not eliminated significantly by the same technique and may be more hazardous than the parent compound. In order to detect the presence of possible remaining estrogenic activity and toxicity of aqueous samples treated by AOPs, bioassays should be used in addition to chemical analytical measurements. In the present study, the removal efficiency of toxicity and estrogenic activity of BPA aqueous samples treated by photolytic/photocatalytic oxidation and CWAO processes in the presence of different TiO_2 - based catalysts was investigated. The efficiency of BPA and TOC removal in treated solutions was investigated by HPLC and TOC analyses, respectively, toxicity tests with bacteria *Vibrio fischeri,* green algae *Desmodesmus subspicatus,* water fleas *Daphnia magna,* zebrafish embryos *Danio rerio* and estrogenicity with yeast estrogen screen (YES) assay. In general the presence of catalysts significantly enhanced the removal of BPA from aqueous samples and consequently diminished toxicity and estrogenic activity of BPA samples treated by both catalytic oxidation processes. The obtained results indicated that removal of BPA was not always well correlated with removal of toxicity and estrogenic activity from treated samples, probably due to production of some toxic and estrogenic intermediates during photolytic/photocatalytic oxidation and CWAO processes. Degradation pathways of BPA in aqueous samples depend on the type of catalyst, advanced oxidation process and experimental conditions used to perform oxidation experiments.

70

Efficiency evaluation of a membrane bioreactor to remove emerging pollutants from a hospital effluent based on the combined use of in vitro and in vivo bioassays and targeted chemicals analyses

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Hospital effluents have been identified as an important source of various chemical classes. Because of potential associated risk for humans and wildlife, elimination of such compounds has become an increasing issue of concern. Conventional treatments are often insufficient to eliminate all the active micropollutants. In particular, chemical diversity of endocrine disrupting compounds (EDCs) limits their removal through targeted approaches. Therefore, new processes have emerged, including membrane bioreactor (MBR) to improve removal efficiencies**.** In the present study, we aimed to evaluate the efficiency of an MBR pilot for the elimination of i) EDCs using a battery of *in vitro* bioassays (i.e. reporter cell lines) covering a panel of endocrine activities, ii) various pharmaceuticals classes using LC-MS-MS analysis, in hospital effluents. In parallel, standardized ecotoxicological tests were performed to characterize ecotoxic potency of effluent before and after MBR treatment. All these tools were deployed on several sampling campaigns. In the non-treated effluent, estrogenic and androgenic activities were quite similar to those previously reported in other hospital effluents whereas GR-and PXR-like activities were close to those found in industrial effluents. After MBR treatment, an overall reduction of the endocrine activities was noted.Target chemical analyses revealed the occurrence of a broad range of pharmaceutical classes including cytostatic anticancer drugs and antibiotics but also metabolites in the non-treated effluent. Overall, concentrations were similar to those reported in other studies, except for very high amount of the antibiotic ciprofloxacin (up to 1mg/L). MBR treatment allowed either total, partial or no removal of targeted pharmaceuticals classes, depending on chemical. Finally, ecotoxicological tests showed effects at several trophic levels in the non-treated effluents. MBR treatment significantly reduces this ecotoxicity. Altogether, our results confirm the relevance of the combined use of biological and chemical analyses for an hoslitic characterization of hospital effluent contamination by a broad range of active chemicals. We report also that this complex mixture leads to global toxicity for exposed organisms at different trophic level confirming hazard for aquatic ecosystems. One major outcome is the ability of MBR treatment to reduce both endocrine disrupting activity and global toxicity although some persisted after treatment

71

Aquatic Ecotoxicology: To Assess the Environmental Performance of Sanitation System on Aquatic environment

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The sanitation system focuses on a variety of pollutants from domestic, agricultural and industrial activities that are potentially a source of pollution in the receiving environment. To determine and assess their potential impact on the ecological quality, this collaborative project between IRSTEA & Veolia Environnement Research & Innovation (VERI) has developed a methodology to measure, monitor and define a battery of discriminating bioindicators. A battery of three biological tools has been selected to assess their sensitivity and pertinence in measuring the potential impacts of a wastewater treatment plant. The tools used are mainly based on the measurement of individual responses (survival, growth, reproduction and feeding) which have a direct link with the dynamic of population's. These tools have been developed on model organisms widely present in our freshwater ecosystems, covering several phyla (insect, crustacean and mollusk). In addition several emerging pollutants as pharmaceuticals were analyzed in the organisms to assess chemical impacts of the sanitation system and inform on their relative sensitivity to exposure . Life history traits - biological descriptors - endure the influence of environmental parameters such as temperature and pollutants. Also, their use in a real environment requires a good knowledge of their behavior

according to the variation of these parameters. To avoid the interference of such parameters, a field laboratory will be installed to reproduce the in situ conditions (pollutant pressures) while controlling the influence of confounding factors. The development of a methodology for the application of biological tests for measuring and monitoring the impact of wastewater discharges will provide a better understanding of wastewater treatment plants' contribution to the water quality of receiving systems subject to multiple pressures.

72

Dioxin-like effect potentials and related effects in fish exposed to regular wastewater effluents and effluents with additional wastewater treatment stages

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The removal of micropollutants like pesticides, pharmaceuticals, and other industrial and household chemicals by regular wastewater treatment is often imperfect. In order to overcome this deficiency, additional treatment stages using ozonation, sand- or carbon-filtering have been included into wastewater treatment during the last years. The project SchussenAktiv*plus* aims at an efficiency control of such additional cleaning methods in three wastewater treatment plants (WWTPs) and two stormwater overflow basins (SOBs) in the Lake Constance area. Within the project, chemical and microbiological analyses and various biological *in vitro* and *in vivo* tests are conducted by several co-operating partners. Two of the three WWTPs under investigation are connected to the Schussen River, a tributary of Lake Constance. A model WWTP has been installed at the WWTP Eriskirch which is located at the Schussen estuary. This model includes effluent-treatment with ozonation, a sand filter, and a carbon filter after the final clarifier of the traditional cleaning stages. Two aquaria were placed at the WWTP for rainbow trout exposure, receiving either water of the actual final effluent after sand filter/flocculation, or water of the model installation. The WWTP Langwiese, Ravensburg, has been equipped with an activated carbon filter which is in operation since September 2013. Cage exposure with rainbow trout was conducted directly in the river upstream and downstream of the effluent before and after the upgrade. For control, rainbow trout were kept in climate chambers. Chemical analysis of water, effluent, and fish samples was conducted. Concentrations of the analyzed PCBs were below limit of detection. Dioxin-like effect potentials were measured by a reporter gene assay using rat hepato-carcinoma cells. Higher potentials were measured in the regular effluent of the WWTP Eriskirch compared to the effluent of the model installation. Concerning the WWTP Langwiese, potentials were detected in the effluent prior to the upgrade. EROD activity in samples from the WWTP Eriskirch was significantly lower in fish kept at the effluent of the model installation compared to the regular effluent. Prior to the upgrade of the WWTP Langwiese higher EROD activity was measured in trout exposed in the Schussen River downstream of the WWTP than in those exposed upstream. Sampling after the upgrade will be conducted in March 2014 and results will be presented at the SETAC Europe 2014.

73

Reduction of micropollutants and pathogens in surface waters by improved wastewater and rainwater treatment: What is the benefit for aquatic organisms and human health?

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The joint research project SchussenAktiv*plus* aims to ascertain the efficiency of various wastewater and rainwater cleaning technologies for the reduction of micropollutants and sanitarily relevant pathogens including multi-resistant bacteria in surface waters with regard to the benefit for aquatic organisms and human health. As a model river in a highly populated catchment area, the river Schussen and, as a reference, the river Argen, two tributaries of Lake Constance, Southern Germany, are under investigation in this project. The cleaning technologies include combinations of ozonation with different sand and charcoal filters, a lamella separator connected to a storm water overflow basin, and a retention soil filter. The quality of these technologies installed at three waste water treatment plants (WWTPs) of different size and type, and at two stormwater overflow basins (SOBs) is assessed by means of ample analytical and microbiological exposure analyses and by extended *in vitro* and *in vivo* effect studies which reflect consequences for biota from the molecular to the community level. Toxic and endocrine potentials in water samples from the five test systems as well as in surface water and sediments of five field sites are assessed by laboratory *in vitro* tests which include reporter gene assays based on yeast and vertebrate cell lines, but also by *in vivo* laboratory studies, as the early life stage test with the zebrafish *Danio rerio* or the growth inhibition tests with *Lumbriculus variegatus* or *Lemna minor*. Toxic and endocrine effects, in contrast, are investigated *in vivo* either in feral chub, spirlin, or gammarids sampled from the field, or in fish, gammarids, and snails actively exposed either to effluents in aquaria or cages, or to the river water in flow-through bypass systems. The impact on the health of fish and invertebrates by toxic and endocrine action is assessed by means of different biomarkers (e.g. histopathological changes, genotoxic effects, stress protein, biotransformation enzyme and vitellogenin induction). Exemplarily, results for chemical, microbiological and biological effect analyses will be presented.

74

Ecotoxicological consequences of pharmaceutical facility discharges on wild fish: a french case of study.

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Discharges of wastewater treatment plants receiving effluents from pharmaceutical factories represent a non-negligible source of biologically active chemicals in surface waters able to induce ecological/ecotoxicological effects on wildlife and populations. Recently, ecotoxicological effects on teleost fish and tadpoles exposed to dilutions of Indian bulk drug manufactures effluents have been concluded, suggesting that deleterious biological effects could be induced. Furthermore, an *in situ* study has demonstrated a high proportion of intersex in gudgeons (*Gobio gobio*) living downstream from a French bulk drug manufacture discharges which could be explained by the nature of pharmaceutical production. The present work consists of an evaluation of long-term adverse effects induced by another French pharmaceutical manufacture discharges in wild fish (*Gasterosteus aculeatus*) using a multi biomarker approach. Ecotoxicological analysis includes a semi-quantitative evaluation of histopathological lesions in liver and spleen, an analysis of intersexe frequencies, DNA damages on erythrocytes, and biochemical markers related to biotransformation capabilities (EROD, GST, CYP 3A) and neurotoxicity (AchE). Our results highlights a marked hepatotoxic effects mainly characterized by fibrosis, lipoid degradation, confirmed by inductions of biochemical responses, increased densities of splenic melanomacrophages aggregates, and elevated DNA damages in sticklebacks sampled in receiving waters. This works argues for the implementation of monitoring programs, using effect-based tools, and the need to improve our knowledge about possible ecotoxicological disturbances occurring in specific waters which receiving pharmaceutical facilities discharges. *This work was supported by the French Ministry for Ecology and Sustainable Development (MEDDE-Program 181).*

Fate and effects of nanoparticles under environmentally realistic conditions (II)

75

Toxicity of silver nanoparticles on natural microbial freshwater communities in an artificial indoor stream

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Data on the toxicity of silver nanoparticles (AgNPs) for individual standard microbial test species are well documented in the scientific literature. However, main drawback of single-species methods is their limited ecological relevance, as it is unknown how the sensitivity of the particular test species towards NPs relates to the sensitivities of the plethora of species that are exposed simultaneously in a natural ecosystem. This study used natural microbial freshwater communities from a stream (Gauernitzbach) near Dresden (Germany), settled on ceramic tiles and transferred to controlled test conditions in artificial indoor streams (AIS). The communities were exposed to two concentrations of AgNPs and $AgNO_3$ (2 and 20 µg/L). Two untreated control channels were used as a reference. Exposure was continous for up to 18 days with four subsequent sampling points (day 0 as a reference point for the communities and 4, 11 and 18 days for an effect analysis). Effects on the algal part of the communities were studied through a pigment profile analysis, the bacterial part of the communities was analysed with metabolic profiling (EcoPlates™). Tolerance patterns at the end of the 18 days exposure period were described using MT2 plates, following the concept of a Pollution

Induced Community Tolerance (PICT) approach. The experiment was supported by comprehensive chemical analysis (AAS, ICP-MS for total and dissolved silver concentrations during exposure/stocks, TEM (particle size, homogeneity and quality of the stocks), NTA (particle size distribution of the stocks), CPS (particle size distribution of the stocks)). Analytical results indicate a rapid dissolutin of the AgNPs, a silver accumulation in the biofilms (higher for the AgNPs than the $AgNO_3$) and a silver concentration decrease in the water phase over time. Ecotoxicological results show differences between the AgNP and the $AgNO_3$ exposed communites with more distinct effects for the $AgNO_3$. Experimental data are currently (November 2013) undergoing final evaluation and modeling, in order to describe and quantify the impacts of $AgNO_3$ and AgNPs on the biofilm biocoenoses in detail. Results will be discussed and presented in relation to the analytical data.

76

Long term effects of cerium dioxide nanoparticles (nCeO2) on a simplified food chain of micro-organisms and zebra mussels

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As the field of nanotechnologies is expanding exponentially, more and more nanomaterials of various physico-chemical properties are being developed for new applications and incorporated into commercial products. The release of significant amounts of those nanomaterials in the environment is expected but the ecological consequences are quite unknown. To answer those concerns, the MESONNET project, headed the French GDRi iCEINT and by the US-CEINT, was set up to evaluate the fate and impacts of nanoparticles within terrestrial and aquatic ecosystems. The project included the building of a network of small mesocosms in three French laboratories, working with the same experimental designs on different species to gather complementary data. We first decided to work on cerium dioxide nanoparticles ($nCeO_2$), mostly used in wood stains and as fuel additives. In the experiment conducted in our laboratory, we investigated in 9 mesocosms the effects of two forms of commercial $nCeO_2$, bare nanoparticles (primary size 3 nm), and citrate-coated nanoparticles (primary size 3 nm) used in wood stains, at low concentration (12 injections of 84 μg/L during 4 weeks - 1 mg/L final) on a simplified ecosystem constituted of a natural bacterial consortium, three green algae species, and an invertebrate, the bivalve *Dreissena polymorpha* or zebra mussel. The fate of nanoparticles and biological effects on the organisms were followed for four weeks. Bacterial and algal biomasses and community structures were determined, while a broad set of immunological, biochemical and functional biomarkers were used to evaluate $nCeO_2$ exposure effects on zebra mussel on different biological targets and at different biological levels. Results were synthesized using the Integrated Biomarker Response (IBR) tool. Internal $nCeO_2$ concentrations and speciation of cerium inside the digestive gland were also measured. Results suggested $nCeO_2$ exert no serious toxicity on bacteria, algae and zebra mussels. We did not see any direct impacts of $nCeO_2$ on micro-organism biomasses while bacterial community structures were modulated. For zebra mussels, while $nCeO_2$ accumulated into digestive gland and was reduced from Ce^{4+} to Ce^{3+}, a transient activation of immune system was observed, but gill mRNA and digestive gland biomarker data showed lower expressions and activities of antioxidant enzymes and a decrease in cellular membrane lipoperoxidation, suggesting a potential antioxidant protecting activity of $nCeO_2$.

77

Silver nanoparticles flow in a model aquatic trophic chain

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Silver nanoparticles (AgNP) are incorporated into a variety of products ranging from personal care products, food packing and medical utilities, serving as an antimicrobial agent (Sotiriou & Pratsinis, 2010). The worldwide estimated production of AgNP is between 250 and 312 tons per year (Hendren, Mesnard, Dröge, & Wiesner, 2011) from which it is still unknown how much silver will end up in the environment. In this study we focused on three species representative of a model aquatic trophic chain, a primary producer (*Pseudokirchneriella subcapitata*), a primary consumer (*Daphnia magna*) and a secondary consumer (*Carassius auratus*), to evaluate the potential food-chain bioaccumulation of AgNP. The final aim of this work was to realize a worse case scenario in which *C. auratus* was exposed to AgNP via water and food. A step-by-step approach was developed in order to find the conditions in which the species of lower trophic levels would concentrate AgNP at a higher rate. *P. subcapitata* was exposed to AgNP and $AgNO_3$ for 48 hours followed by a 48-h elimination period. AgNP accumulation in *D. magna* was studied upon exposure through water, food and both water and dietary exposure. Finally, the worse case scenario of AgNP accumulation in *D. magna* was chosen to produce a model trophic chain in which *C. auratus* was exposed for 10 days to both AgNP and $AgNO_3$ contaminated water and food (*D. magna*) followed by 7 days of elimination. Following exposure of *P. subcapitata* to both AgNP and $AgNO_3$, the algae did not internalize AgNP but did accumulate Ag ions. *D. magna* accumulated higher amounts of Ag when exposed through water and food, and also had higher Ag body burdens when exposed to AgNP than to $AgNO_3$. The pattern of Ag bioaccumulation in *C. auratus* differed between AgNP and $AgNO_3$. In conclusion, our data suggests that AgNP have the potential to bioaccumulate within an aquatic trophic chain, which may be due to their physico-chemical properties and the interaction with biological tissues.

78

Habitat selection of a leaf shredding amphipod affected by photocatalytic properties of nTiO2

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The increasingly worldwide use of titanium dioxide nanoparticles ($nTiO_2$) raises the concern of potential risks for wildlife when released into the aquatic environment. For this reason, numerousness studies focused on their toxicity towards aquatic wildlife, however, mostly assessing the sole effects of $nTiO_2$. Consequently, there is only limited information about ecotoxicological effects of $nTiO_2$ in combination with environmental parameters. The present study investigated the (in)direct effects of $nTiO_2$ towards the amphipod *Gammarus fossarum* (Crustacea: Amphipoda), considering the photocatalytic properties under environmentally relevant intensities of UV-A and UV-B irradiation. Gammarids' habitat selection was thereby used as a sublethal measure of response, judged by their feeding preference on leaf discs either exposed to or protected from direct UV-irradiation. UV-protected parts of the test vessel were significantly preferred ($p < 0.001$) when gammarids were solely exposed towards UV-irradiation, indicated by approximately 40% less feeding in the part of the test vessel directly exposed to UV-irradiation compared to the UV-protected counterpart. Most likely, an avoidance of the damaging effects of UV-irradiation for amphipods may explain this behavior. The same significant habitat selection pattern ($p < 0.001$) was observed when both $nTiO_2$ and UV-irradiation were applied as stressors, however, with up to 85% less feeding on UV-exposed leaf discs. This two-fold increase in feeding preference may be explained by the photoactivation of $nTiO_2$ since no such pattern was observed for $nTiO_2$ alone: the photocatalytic properties led to the formation of reactive oxygen species (ROS) under UV-irradiation, provoking an active habitat selection of gammarids to diminish deleterious effects (e.g. lipid peroxidation). On the other hand, ROS may have influenced the nutritional value of leaf discs. Gammarids may have thus rather preferred a higher quality food source due to its palatability, explaining the observed habitat selection (=indirect effect). Summing up, results of the present study clearly demonstrated environmental parameters (represented by UV-irradiation) to influence the ecotoxicity of $nTiO_2$. The understanding of interactive effects between nanoparticles and environmental parameters seems therefore fundamental for an accurate prediction of environmental risks posed by nanoparticles, which should be further investigated in the future.

79

Individual and functional impacts of environmentally realistic silver nanoparticle exposures on Gammarus sp (Amphipoda)

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By means of some antibacterial properties, silver nanoparticles (nAg) are more and more widely used in current consumer products and probably released into aquatic environment. One of the main priorities is now how to perform proper evaluation of risk using environmentally realistic exposure concentrations and media. Gammarids are currently used as test organism in ecotoxicology. They also play a major role in ecosystem functioning by their capacity to breakdown leaf-litter. The fine particulate organic matter (FPOM) released by the shredding activity of *Gammarus* can be in turn incorporated in the aquatic food chain. In this context, the effects of low concentrations (from 0.5 to 50 μg/l) of silver nanoparticles with

different sizes, shapes and coatings were tested on *Gammarus sp*. The first study, conducted in microcosm systems, aimed to assess the individual and functional impacts on three *Gammarus* species during three days. Then, most sensitive species was studied during three weeks in a more complex medium (mesocosms) containing sediment and other freshwater organisms (microorganisms, invertebrates). Our objectives are to use various endpoints at different biological organization levels from individual (physiological, behaviour) to ecosystemic ones. Realistic silver concentrations did not induce mortality of gammarids despite a metal bioaccumulation. All nanoparticle and nitrate silver exposures induced a significant locomotion decrease. These impacts were dependant of the nanoparticle size, shape, concentration, exposure time and medium. The effects were also more important for *Gammarus roeseli* which seemed to be the most sensitive species. For example, leaf litter consumption and FPOMs production were significantly reduced by the 10nm silver nanoparticle at 5 μg/l. On the contrary, the assimilation was really increased. This increase could be interpreted as an indirect effect: exposure may conduct organisms to enhance energy storage in order to be able to face energy cost when detoxification will be needed. For environmentally realistic concentrations, silver nanoparticle contamination has direct effects on organism's behaviour and function. These impacts are dependant of the size and the shape of nanoparticle. Interestingly, we found that low nAg concentrations, through the effects on the energetic metabolism of *G.roeseli* (increased assimilation) may cause indirect effects on the aquatic organic matter cycle.

80

The mesocosm approach to estimate the fate, bioaccumulation and toxicity of silver nanoparticles on two endobenthic species: the bivalve mollusk Scrobicularia plana and the worm Nereis diversicolor

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The widespread use of engineered silver nanoparticles (Ag NPs) will likely lead to their increase into the environment. The aim of this study was to evaluate under environmentally realistic conditions the fate, bioaccumulation and toxicity of maltose-Ag NPs on clams (*Scrobicularia plana*) and worms (*Nereis diversicolor*). Animals were exposed in outdoor mesocosms to Ag (10 μg.L^{-1}) added either in the nanoparticulate or soluble forms in comparison with controls for 21 days. The fraction of Ag under labile forms was determined in water and sediment by using Diffusive Gradient in Thin films (DGT). Bioaccumulation of Ag was quantified in the whole soft tissues of both species. Toxic effects were evaluated using a set of biomarkers at different levels of biological organization: i) sub-individual level (biochemical biomarkers) and ii) individual level (behaviour, feeding rate). Results from DGTs indicate a release of Ag ions from Ag NPs either in seawater as in sediment. Both Ag forms were bioaccumulated in both species but no differences between forms were shown. This result is not surprising since a solubilization of Ag from Ag NPs was observed. At the sub-individual level, GST, CAT and CSP activities were induced in both species exposed to soluble and Ag NPs compared with controls. Greater PO and lysozyme activities were observed in clams and worms respectively, exposed to Ag NPs compared to other treatments. DNA damages in the digestive gland of clams were higher in contaminated Ag NPs mesocosms compared to soluble Ag and controls. These results suggest that dissolved Ag species partly control toxicity effects but cannot alone completely explain them, suggesting a specific nano effect. At the individual level, soluble Ag only induced burrowing impairments in both species compared with Ag NPs and controls. This is the first experiment with endobenthic species exposed to Ag as soluble and NP forms under environmentally realistic conditions. Such a degree of complexity in term of exposure to engineered NPs and of the related impacts is a real challenge for the scientific community, and will provide necessary data for Environmental Risk Assessment. This work has received funding from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement No. 214478 (NanoReTox). Key Words: silver nanoparticles, outdoor mesocosms, *Scrobicularia plana*, *Nereis diversicolor*

Human exposure to emerging contaminants: monitoring and modeling (II)

81

Accounting for volatilization losses of dermally applied cosmetic ingredients: How important is the inhalation route?

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Accurate modeling of aggregate consumer exposure to a cosmetic ingredient is essential for its safety assessment. For many personal care products that are applied dermally, exposure to their ingredients is considered to occur mainly via dermal absorption, unless vapor pressure of a substance is above a certain cut-off value. However, the contribution of inhalation to the total systemic dose of a substance may potentially be affected by other factors influencing the substance's evaporation from skin, e.g. interaction with other ingredients contained in a product, also called matrix effect (Jovanovic et al., 2008; Tokarzcyk et al., 2012) or binding to lipids/proteins in viable epidermis. In order to test the effect of the product matrix on the volatilization of a common cosmetic ingredient, decamethylcyclopentasiloxane (D5), which is widely used in personal care formulations, we compared the volatilization of the neat substance and two different commercially available formulations: a deo roll-on and a face cream. Single doses of D5 were applied neat (control) and in formulations on either an aluminum circle or porcine full-thickness skin *in vitro* resting on a modified static diffusion cell at two working temperatures (23°C and 32°C). The masses of D5 remaining on the surface, D5 penetrated into the receptor fluid, D5 captured in the trap and D5 retained in the chamber were measured after 15, 30, 45, 60 and/or 75 minutes of exposure. Quantification of D5 was done based on the internal standard (M4Q) quantification procedure using GC/FID, following D5 extraction/desorption. The evaporation rates were determined as slopes of the regression lines for the volitilized mass over time adjusted for the surface area exposed. The results were analyzed using a one-way blocked analysis of variance. The cosmetic formulation had a significant effect on the evaporation rate of D5 (F-test=27.9, p-value< 5.4e-08). The differences in mean evaporation rates between the groups were all significantly different from zero. The greatest difference was observed for the neat-face cream pair at 32°C. No significant difference in evaporation rates was observed between aluminum and skin surface. Overall, the volatilization of D5 from skin *in vitro* occurred very fast, thus indicating that indirect inhalation exposure to volatilized material is a very important exposure pathway. Other ingredients contained in a cosmetic formulation can substantially lower the evaporation rate of D5.

82

Traffic-Related Metal Emissions and their Bioaccessibility in Urban Atmospheric Dust of Human Health Concern

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Metal(loid)s emitted from automotive traffic are suspected contributors to the negative health effects observed among urban populations exposed to respirable fractions of atmospheric dust. While measures of total metal(loid) concentrations in urban particulate matter (PM) are important, more information regarding elemental bioaccessibility is essential to support assessments of risk. This study used artificial lysosomal fluid (ALF) and Gamble's solution to simulate conditions in the human lung to assess metal(loid) solubility in airborne PM_{10}, $PM_{2.5}$ and PM_1 sampled in Frankfurt am Main, Germany. Samples, collected using a high volume sampler between June 2009 and November 2010 at a busy roadside location (n=43), were incubated with the simulated lung fluids at 37ºC. The extracts were analyzed for a range of metal(loid)s, including As, Ce, Co, Cr, Cu, Mn, Ni, Pb, Sb, Ti, Pt, Pd and V using an ICP-Q-MS (Varian 820-MS). Metal(loid) concentrations were also determined for filters digested with aqua regia (HNO_3/HCl ratio: 1:3). The results demonstrate that many traffic-related metal(loid)s are highly soluble in simulated lung fluids. In particular, Cu, As, Pb, V and Sb were observed to be the most soluble. Metal(loid) solubility was observed to be strongly pH dependent, with the highest solubility observed for samples extracted with the acidic ALF. Further, metal(loid) solubility did not always increase with decreases in particle size fraction, as is often predicted. For instance, the solubility of Pb was observed to decrease with increasingly finer PM fractions, with a mean solubility of 96 and 78% in PM_{10} and PM_1, respectively. The results demonstrate that many of the toxic metal(loid)s associated with airborne PM are soluble in lung fluids, increasing the risk of negative health outcomes in exposed individuals. Given the complex mix of airborne PM, risk assessments will ultimately need to examine the combined effects of contaminant exposures.

83

Inverse age-dependent accumulation of decabromodiphenyl ether and other PBDEs in serum from a general adult population

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Polybromodiphenyl ethers (PBDEs), including the decabromodiphenyl congener (BDE-209), were determined in serum of 731 individuals from a general adult population (18-74 years) collected in 2002 in Catalonia. The BDE-209 was the predominant congener (median 3.7 ng/g lipid) followed by BDE-47 (2.6 ng/g lipid) and BDE-99 (1.2 ng/g lipid). PBDEs in this population (median 15.4 ng/g lipid) ranked among the highest of previously described concentrations in populations in Europe, Asia, New Zealand and Australia, yet they were lower than those found in North America. Age was clearly the socio-demographic factor of highest influence

on the PBDE distributions. However, unlike usual trends of higher accumulation of POPs through age, the higher concentrations were found in young individuals (< 30 years) rather than in adults (≥ 30 years), with differences of 14%, 31% and 46% in the most abundant congeners (i.e. BDE-209, BDE99 and BDE-47, respectively). This age-dependent distribution of PBDEs (including the case for BDE-209, which is shown for the first time in this cohort) is explained by the higher and widespread use of these compounds since the 1980s. In view that these compounds remain highly used, this accumulation pattern is likely to evolve, anticipating an increasing level of PBDE concentrations in future general population surveys, yet probably assuming an age-dependent increase pattern. Socio-economic level was also a determinant of BDE-47 concentrations, but only relevant for the least affluent class, suggesting that lifestyle and environmental conditions in the dwelling place may also contribute to exposure. Nonetheless, gender, body mass index, place of birth, parity and education level did not show any statistically significant influence on the observed PBDE distributions.

84

Determination of DEHP metabolites, PFOS and PFOA in breast milk and cord plasma samples from European birth cohorts

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The incidence of childhood obesity has reached epidemic proportions globally. There is accumulating evidence that factors that influence long-term risk of obesity and related disorders begin very early in life. Early life exposure to environmental contaminants has been implicated in altering developmental programming, resulting in possible higher susceptibility to obesity. The OBELIX (OBesogenic Endocrine disrupting chemicals: LInking prenatal eXposure to the development of obesity later in life) project examined the hypothesis that prenatal exposure to endocrine disrupting compounds (EDCs) in food plays a role in the development of obesity later in life. The project focused on assessing prenatal exposure to chemicals from six major classes of EDCs found in food including dioxins and dioxin-like polychlorinated biphenyls (PCBs), non-dioxin-like PCBs, brominated flame retardants (BFRs), organochlorine pesticides, phthalates and perfluorinated alkyl acids (PFAAs), e.g. perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS). In this presentation, method development, validation and analytical results of the quantitative trace level analysis of DEHP metabolites, PFOS and PFOA in cord plasma and breast milk samples from different European birth cohorts (FLEHS II, HUMIS, Michalovce, Linc) will be discussed. The levels of PFOS, PFOA and selected secondary DEHP metabolites in cord plasma and breast milk will be compared with those obtained in other cohort studies. However, especially for secondary DEHP metabolites in cord plasma, data are extremely scarce, emphasizing the uniqueness of our study. Other topics to be addressed are e.g. the influence of contamination of the samples and remaining enzymatic activity on the suitability of specific metabolites for exposure assessment

85

Nontarget analysis of organic pollutants in human urine: developing a generic screening method

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Human biomonitoring programs usually analyse human samples for a limited number of chemicals and biomarkers. However, there might be many more chemicals of concern to human health, which so far are unknown or simply not analysed for so far. The goal of this project is thus to develop screening methods for the detection of new emerging chemicals in human tissues like urine and blood. Here we present the method development for a nontarget screening of human urine. 38 analytes representing different chemical groups with a wide range of properties were chosen for recovery experiments and determination of lowest detectable concentrations. Direct injections of spiked samples into a liquid chromatograph coupled to a high resolution mass spectrometer and sample preparation via QuECheRS (Quick Easy Cheap Rugged and Safe) extraction were tested. Recoveries show that by a combination of both sample preparations all analytes can be detected. Thus this type of sample preparation is suitable for a nontarget screening of human urine samples. The additional application of a deconjugation step makes it possible to detect the original substances and thus have a control for the nontarget detection of metabolites. The nontarget screening of a set of human urine samples will be presented at the conference.

Advancing Adverse Outcome Pathways for Integrated Toxicology and Regulatory Applications (II)

86

SeqAPASS: Sequence alignment to predict across-species susceptibility

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Efforts to shift the toxicity testing paradigm from whole organism studies to those focused on the initiation of toxicity and relevant pathways have led to increased utilization of in vitro and in silico methods. Hence the emergence of high through-put screening (HTS) programs, such as U.S. EPA ToxCast, and application of the adverse outcome pathway (AOP) framework for identifying and defining biological key events triggered upon perturbation of molecular initiating events and leading to adverse outcomes occuring at a level of organization relevant for risk assessment [1]. With these recent initiatives to harness the power of "the pathway" in describing and evaluating toxicity comes the need to extrapolate data beyond the model species. Sequence alignment to predict across-species susceptibilty (SeqAPASS) is a web-based tool that allows the user to begin to understand how broadly HTS data or AOP constructs may plausibly be extrapolated across species, while describing the relative intrinsic susceptibiltiy of different taxa to chemicals with known modes of action (e.g., pharmaceuticals and pesticides). The tool rapidly and strategically assesses available molecular target information to describe protein sequence similarity at the primary amino acid sequence, conserved domain, and individual amino acid residue levels. This in silico approach to species extrapolation was designed to automate and streamline the relatively complex and time-consuming process of comparing protein sequences in a consistent, logical, and criteria driven manner intended for predicting across species susceptibility to a chemical perturbation. To define the domain of applicability and enhance the utility of the SeqAPASS tool, multiple case studies have been explored, including the derivation of predictions for across species susceptibilty to chemicals that target the human estrogen receptor, bovine androgen receptor, mosquito voltage-gated sodium channel, fungus cytochrome P450 51, and honey bee nicotinic acetylcholine receptor. These examples highlight the utility of the SeqAPASS tool for researchers and regulators alike.

87

Predicting the sensitivity of endangered sturgeons to dioxin-like compounds: Molecular investigation into the aryl hydrocarbon receptor pathway

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Numerous sturgeon species around the world are endangered, which has rendered these fishes of interest in context with the risk assessment of anthropogenic stressors. However, current risk assessment attempts are hampered by a lack of knowledge about the sensitivity of sturgeons to toxicants of concern, such as dioxin-like compounds (DLCs). DLCs elicit their toxic action through activation of the aryl hydrocarbon receptor (AhR) and are known to cause a variety of adverse effects in vertebrates. In birds, the amino acid sequence of the ligand binding domain of the AhR is known to result in differences in affinity for DLCs and therefore differences in sensitivity among species. In order to incorporate differences in species sensitivity of sturgeons to DLCs into the adverse outcome pathway framework, the objectives of this study were to: 1) characterize the relative differences in sensitivity to DLCs between two species of endangered sturgeon (white sturgeon and lake sturgeon) and 2) develop methods to predict the relative sensitivity to DLCs of other species of sturgeons based upon knowledge of species differences in mechanisms of the molecular initiating event. White sturgeon were found to have 10-fold greater response following exposure to a model DLC, compared to lake sturgeon with regard to up-regulation of hepatic CYP1A transcript abundance. Since effects of exposure to DLCs are known to be driven by activation of the AhR, the AhR1 and AhR2 of white and lake sturgeon were cloned and characterized in an attempt to explain the observed differences in *in vivo* responses between these species. White and lake sturgeon AhR1s had similar sensitivity to 2,3,7,8-TCDD *in vitro* using the luciferase reporter gene assay, while white sturgeon AhR2 had an EC50 10-fold less than lake sturgeon AhR2. Relative differences in response between white and lake sturgeon *in vivo* and with regard to AhR2 activation *in vitro* appear similar indicating that the AhR2 might drive differences in sensitivity to DLCs among these species of sturgeons. Homology modeling will be used to predict whether amino acid differences in the ligand binding domain of the AhR2 drives the observed differences in sensitivity between the AhR2 of white and lake sturgeon and could allow prediction of the sensitivity of other endangered sturgeon species based on their AhR2 amino acid sequence. This research could be essential in the risk assessment of sturgeons, and other endangered fishes, to DLCs.

88

Application of genome-wide transcript profiling to elucidate the mechanisms of toxicity of environmental chemicals in model and non-model fish species

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In recent years, advances in sequencing technologies and approaches for transcript profiling in biological samples have allowed the unbiased assessment of the mechanisms of toxicity of environmental chemicals in model organisms, mainly through the use of gene expression microarrays. However, the development of genome wide microarrays for non model organisms required very significant research effort, which precluded their use in the majority of environmentally relevant species. As a result, researchers have investigated mechanisms of chemical toxicity principally in model species and datasets for other species of ecological importance are still scarce. The very recent accelerated development in high throughput sequencing technology has allowed for genome wide transcript quantification to be conducted without previous sequencing information and provided researchers with new opportunities to investigate chemical toxicity in both model and non model organisms of ecological relevance. These datasets provide essential information for the construction of adverse outcome pathways to support risk assessment. We have made use of both gene expression microarrays and high throughput sequencing (RNA-Seq) to conduct transcript profiling in model and non model fish species in order to investigate the mechanisms of chemical toxicity in a range of environmentally relevant chemicals (including oestrogens, metals and pesticides) and determine the molecular pathways most affected by each chemical. We observed conserved molecular pathways of response to a number of environmental chemicals and in particular for oestrogens. In addition, our research identified novel effect pathways for a range of chemicals and most notably a striking down-regulation of the cholesterol biosynthesis pathway following exposure to both copper and linuron. These results provide case studies for the utility of global transcriptomic approaches in the identification of the pathways of effect of environmental chemicals in an unbiased manner. In addition, our data provide critical examples of conserved response pathways across vertebrate species.

89

Development of an in vitro metabolomic approach for enhanced neurotoxic effects by contaminants, with emphasis on neurotransmitter pathways

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Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. From a scientific point of view, there is no doubt that exposure to neurotoxic chemicals during early brain development can adversely affect learning and development. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants during human development can have deleterious effects on cognitive development in childhood. The European commission-funded project DENAMIC "Developmental Neurotoxicity Assessment of Mixtures in Children" investigates neurotoxic effects (e.g. learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. One of the aims is to develop better and sophisticated tools, procedures and testing methods to screen compounds for (developmental) neurotoxicity and to improve assessment of exposures and effects (www.denamic-project.eu). As part of the project, a new alternative assessment strategy based on a combination of in vitro and in vivo assays is under development in order to prioritize compounds for further in vivo testing. To this end, hazard characterization of pesticides and environmental pollutants on a molecular and cellular level is carried out, with emphasis on adverse effects during neuronal development. An array of in vitro assays is used to investigate (developmental) neurotoxic effects, including neuron differentiation in the SH-SY5Y human neuroblastoma cell line, acetylcholinesterase (AChE) inhibition, and transthyretin (TTR) binding. An important aspect is the development of biomarkers for (developmental) neurotoxicity in animal models using (epi-)genomics, proteomics and metabolomics. This paper present the development of a metabolomic and neurotoxicity approach for the SH-SY5Y cell line. The focus is on four neurotransmitter pathways: Dopamine, Serotonin, Gaba, and Acetylcholine. Analytical methods were developed to detect and quantify the precursors, neurotransmitters and metabolites in the SH-SY5Y cells using a 12 well based system and LC-MS/MS. An LC-HRTOF system was used for untargeted analysis. SH-SY5Y cells were exposed to various pesticides, their metabolites and MeHg to investigate the effects on the neurotransmitter pathways.

90

From Laboratory to assessing efficiency of Water reprocessing plants

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Anthropogenic contaminants from waste water treatment plants (WWTPs) contribute to the pollution of surface waters. Although conventional WWTPs improve water quality, additional steps such as sedimentation ponds, reed beds and wetland forests can be used before discharge to surface water and have been shown to improve water quality indicators such as dissolved oxygen. The efficiency of the purification process can be assessed by measuring chemical concentrations at different stages of the process. This approach however is limited by its relevance to the biological effect and is limited in scope of chemicals which can be measured. Traditionally ecotoxicology has assessed biological effect through a number of acute or chronic toxicity endpoints and a small number of endocrine disruptor biomarkers. While these have shown to be informative, they suffer from several limitations. They do not for example allow for identification of potential adverse outcome pathways (AOPs) and they do not provide quantitative assessment of risk nor do they allow prediction of exposure effects. To address these limitations we have developed a battery of computational modelling tools and applied these to model a set of control experimental exposures to predict exposure effects in the environment. We analyse an integrated dataset of 1) laboratory exposures to define baseline measurements for 10 different chemicals and 26 complex mixtures and 2) in a mesocosm experiment linked to effluent purification steps at three different WWTPs. Our approach uses and integrated computational biology approach to utilize all available transcriptomic, bioassay and chemistry information on the experiments in a network inference approach linked to predictive modelling methodologies. AOPs defined by this procedure represent inferred networks linking genes and additional measurements and endpoints recorded as part of the experimental design. Here we show its effectiveness in real world applications – assessing the efficiency of WWTPs. While this still only focuses on a relatively small number of compounds we have shown that AOPs can be identified which relate to single and mixture responses and are able to link these in real world applications. Furthermore we show that mixture exposure can lead to additional functions being perturbed.

91

Hepatic vitellogenin induction in fathead minnows is influenced through both soluble estrogen receptors and membrane receptors

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Adverse outcome pathways are defined by linking the interaction of a toxicant with a molecular initiating event and then to a series of steps at increasingly higher biological relevance that lead to a final adverse consequence for the organism. Important for this concept to work is defining precisely the interactions in the pathway, so that there is a clear consequence of exposure to the toxicant. In the case of estrogens, they are believed to bind directly and specifically to soluble estrogen hormone receptors which are then able to bind to response elements in promoters of susceptible genes. Vitellogenin (Vtg) has emerged as a strong biomarker for estrogenic chemicals, induced through soluble estrogen receptors. Male fathead minnows (*Pimephales promelas)* were exposed to 5 ng ethinylestradiol (EE_2)/L or to a mixture of 5 ng EE_2 and 100 ng ZM189,154/L (a potent antiestrogen, known to block activity of all estrogen receptors) for 48 h. Microarray analysis of hepatic gene expression changes indicated, as expected, that many genes, including the three vitellogenins, were up regulated by the exposure to EE_2. Inclusion of ZM189,154 in the mixture blocked expression changes for some genes, but enhanced expression of others, generating two distinct expression patterns. Among the genes that were blocked by the antiestrogen were Vtg 2 and Vtg3, however the mixture enhanced expression of Vtg1, among other transcripts that appear to be targeted via non-genomic signaling pathways. To verify whether the membrane estrogen receptor GPER could influence expression of Vtg 1, fathead minnows were exposed to 5, 30 and 100 ng G1/L, a specific agonist of GPER that does not agonize soluble ERs. Vtg1 mRNA expression increased in a dose-responsive manner in both females and males, suggesting that non-genomic signaling may indeed be involved in regulating the expression of this transcript. Our results suggest that both genomic and non-genomic pathways influence Vtg expression and that membrane receptor molecular initiating events should be considered when defining adverse outcome pathways resulting from exposures that interrupt Vtg synthesis in fish.

Modelling techniques for future-oriented LCA and forecasting scenarios (II)

92

Accounting for resource functionality in resource use impact assessment

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Life Cycle Assessment (LCA) is used increasingly over the past years in decision making, in order to predict environmental impacts of products, processes or services. Hence, LCA is used to asses the environmental benefits of lifestyle changes such as the electrification of the transport sector that is undertaken to diminish the emissions of greenhouse gazes (GHG). Yet, while GHG emissions are diminished, other environmental concerns arise, such as the depletion of the resources needed in electrical cars. Electrifying the transport sector implies an intensive use of resource and questions arise concerning future resource availability: is there enough mineral resources to support a global electrification of transport an all the other human needs given the fact that some of those resources are non substitutable for some functionalities whereas other have easy alternatives. Facing the challenge of linking current LCA with future scenarios is not possible with existing life cycle impact assessment methods. In this study, an approach based on the competition that exists for resources is proposed by accounting for resource availability variation in the future. As variation in a resource's availability affects its end-use users differently, resource is no longer considered for its nature but for the functions it provides. Competition evolves in time depending on the adaptation capacity of its users facing availability decrease. The easier it is to replace a resource or a technology whilst remaining functionally equivalent, the quicker users will adapt and resource competition will decrease accordingly. The consequence of a decreasing availability is a shown by a non-dimensional competition factor, the MAterial Competition Scarcity Index (MACSI), which represents the proportion of the users that will remain unable to fulfil their functionality when the resource is depleted. The moment when depletion occurs is given by the ratio of reserve over current production. The proposed competition factors are obtained for resources used in electrical vehicles batteries and express how fierce competition may be for these resources in the future based on today knowledge. The developed indicator is applied to a case study modelling the potential environmental consequences and benefits that will occur if the province of Quebec reaches its goal of replacing 20% of its current intern combustion engine cars by electrical vehicles.

93

Environmental consequences of stormwater harvesting for flood prevention

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Rainwater has been mostly studied as an opportunity for those regions facing water scarcity problems that will become more severe in the near future. As a result, different strategies were developed in order to reuse rainwater in households, buildings and neighbourhoods. However, it must be taken into account that evacuation systems also play an important role in water and wastewater management, given that intense rainfall might cause flooding events and consequently reversible and irreversible material and human losses. Therefore, the goal of this study is to determine the net environmental benefits of constructing flood prevention systems from a consequential life cycle assessment (CLCA) perspective. Two different scenarios with and without prevention systems are compared and the environmental amortisation of the infrastructure is accounted for considering the production of new cars and new buildings after a flooding event. In addition, the environmental savings deriving from the rainwater reuse will be discounted from the burdens associated to the water supply network. As case study, the city of São Carlos (Brazil) is considered, given the heavy precipitation events that usually take place through the year.

94

CONSEQUENTIAL LCA TO ASSESS ENVIRONMENTAL BENEFITS OF SMART GRIDS

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Development of smart grids is considered as promising to reduce environmental impacts associated to energy consumption. The introduction of communication and information technologies would ensure an optimal adjustment between means of production (including intermittent means as renewable energies), and electrical consumption, thus stabilizing the balance of the grid and reducing the use of the most pollutant plants. This would result in environmental benefits, which still need to be evaluated. Given the time scale and the underlying decision involved, Consequential-LCA was identified as the most relevant methodology to evaluate environmental benefits associated to a Smart Grids project. The identification of the marginal mean of production is based on a forcasting methodology linked to the Spot market price. This methodology has been tested on several management strategies applied to a stock of commercial buildings and industrial sites, in the PACA region (France) in the Reflexe project. This approach allowed identifying the global environmental consequences of a smart management of different power consumption profiles, implemented at local level.

95

The role of dynamic perspectives to model future scenarios for attributional and consequential life cycle assessments: case of old scrap aluminium

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Cities have become huge reserves of materials in form of in-use stock and in subsequent years, this in-use stock will be an important source of waste materials to treat. Therefore, in order to project future scenarios for waste management, dynamic perspectives accounting for this in-use stock should be incorporated into the analysis. Besides, these projections are the basis for determining the future potential GHG benefits of recycling. In this regard, two different methodologies are applied to determine the GHG benefits from recycling, which include attributional life cycle assessments (ALCA) and consequential life cycle assessments (CLCA). As the methodologies are based on different assumptions, results between the two differ. The aim of this study is to show the necessity of incorporating dynamic material flow analysis (dMFA) in LCA (ALCA and CLCA) in order to properly project waste generation trends. As case study, the old scrap aluminium generated in Spain is considered. The projection shows a significant increase in old scrap generation with a value of 290,154 tonnes in 2020, equivalent to an increase of over 780% since 1995 and over 146% since 2010. GHG savings conducted with CLCA double results with ALCA. Main differences are due to the energy mixes profiles considered. In this sense, we have used the average energy mix and average technology mixes in the ALCA, independently of the market constraints or conditions. However, demand for primary aluminium has been displaced to Asian countries, as well the demand for old scrap, which leads to different energy mix profiles and technologies. In this sense, with CLCA it is possible to evaluate the primary aluminium traded in the global market and evaluate changes in supply and demand. If LCA studies on waste management are conducted for decision making, projections should consider the past and current consumptions in order to project proper scenarios. Otherwise, the results could be underestimated.

96

Influence of initial conditions of agent-based model simulation for consequential LCA of bioenergy

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Consequential LCA (C-LCA) aims at identifying the changes (Δ), in terms of potential environmental impacts induced into a system, caused by human driven actions, most often related to policy or strategic decisions. The use of a formal modelling strategy is often advocated to compute Δ more accurately than using simplified scenario-based approaches. In this presentation we deal with the implications of using agent-based modelling (ABM). Our objective is to build an ABM of the consequential effects of possible incentive mechanisms set up to increase the area under maize in Luxembourg to produce biofuels. For this, it is necessary to model farmers' behaviour and market mechanisms of price discovery. Considering behavioural aspects in farming would potentially improve the modelling power. When developing an ABM, one is faced with the problem of initializing parameters. The question we focus on is: what is the sensitivity of simulation results to the initial distribution of agent attributes? This presentation discusses using ABMs for C-LCA and the influence of using different strategies to initialize values of the ABM. In our model, agents have a profit maximizing behaviour. Specification of past prices is known to all farmers and a common forecast of future price of each crop. We have implemented the model in a virtual laboratory programmed in java. Two different approaches were used to initialize parameters. One is to distribute them uniformly around the mean for each farm type. The other is to fit a right-skewed distribution to the entire farming sector with a global average farm size. The resulting values are similar in their means and standard deviations, when compared farm class wise, but the number of farms belonging to each farm class is different. The results achieved so far show that the modelling strategy followed is suitable to address the complexity of the problem at hand for Consequential and future-oriented LCA.

97

Towards comparative short-period time-resolved life cycle assessment of differently charged electric vehicles

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Local traffic emissions, like noise, particulate matter, ozone or nitrogen oxides are a threat to human health in cities worldwide. Many municipalities try to abate those emissions by restricting vehicle access and promoting electric mobility in the city centers, which can reduce overall emissions or shift emissions to less occupied regions. Not only individual car-traffic is responsible for those emissions. Large vehicles, such as public transit buses, could also be electrified in order to reduce the local traffic emissions. Several energy storage system options for powering such electric vehicles are available on the market, e.g. batteries or supercapacitors. Supercapacitors, which have a smaller energy density but longer service lifetime than lithium-ion batteries, are expected to be frequently recharged while the bus is in operation. Whereas batteries are expected to be recharged when the vehicle is off duty. This means that a supercapacitor-bus is quickly recharged at bus stops and has only a short driving range between two halts, while in contrast a battery is recharged over several hours in the depot and can propel the bus over the full operation range on a single charge. Thus the energy storage type determines the charging timeframe. It is expected that the energy supply varies during the operation and non-operation periods of the vehicle and thus the charged energy of both storage types has different environmental impacts. For example, the supercapacitor-vehicle is expected to be mostly charged during daytime, when more environmentally friendly photovoltaic electricity is available in the power grid. In order to perform a comparative life cycle assessment of both energy storage types, a detailed analysis of the power supply is necessary. In this study the hourly and seasonal variation of the German electricity mix is used in a time-resolved manner in order to calculate the different environmental impacts of on-duty and off-duty charging of electric public transit vehicles. Time-resolved electricity mix data is taken from public sources with minimal use of generic models. For this first comparative approach the vehicle's and storage systems' production, as well as the infrastructure and the end of life are disregarded. The study is meant as a first attempt to sample time-resolved life cycle assessment for comparative analysis on a small timescale and is thus also aiming at pinpointing issues and development potential of the methodology.

How can scientific advances support regulatory risk assessment for pesticides? (II)

98
Re-evaluation of the assessment factor for the earthworm Tier 1 risk assessment of plant protection products
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The conservatism in tier 1 earthworm risk assessment for plant protection products (PPP) under the Regulation 1107/2009 is expected to increase due to revision of the PEC_{soil} modeling procedure in the near future. EFSA proposes to use realistic worst case PEC_{soil} values for each European regulatory zone considering a lower soil bulk density, a lower organic carbon content, and a reduced crop interception rate due to consideration of worst case wash-off assumptions. Furthermore, several different soil layers for which PEC_{soil} values could be calculated are under discussion, i.e. 0-1 cm, 0-2.5 cm, 0-5 cm, and 0-20 cm soil depth. PEC_{soil} values based on the new EFSA proposal are estimated to increase up to a factor of 40 which would lead to an overly conservative tier 1 risk assessment in case these PEC_{soil} values are simply plugged into the current risk assessment scheme with an assessment factor of 5. In order to re-calibrate the future earthworm tier 1 risk assessment, under revised PEC_{soil} modeling assumptions, effect levels in the standard laboratory reproduction tests are compared with the effect levels in higher tier studies for a representative set of case studies compiled by ECPA companies (n=59). For re-calibration of the tier 1 risk assessment, the relevant soil layer for the risk assessment and the assessment factor is adjusted in the way that the regulatory acceptable concentration or rate based on the tier 1 laboratory study (no-observed effect level divided by the assessment factor) does not exceed the NOEAEC or NOEAER derived from the corresponding field study. The results of this evaluation give clear evidence that considering a layer for PEC_{soil} modeling of 0-5 cm in combination with the current assessment factor of 5 would lead to an appropriate earthworm tier 1 risk assessment. Whereas, PEC_{soil} values based on a soil depth of 0-1 cm in combination with an assessment factor of 5 would lead to an overly conservative tier 1 risk assessment. This would trigger a large number of additional field studies without any indication for a real-world problem. It is therefore recommended to leave the relevant layer for PEC_{soil} modeling at 0-5 cm in combination with a tier 1 assessment factor of 5 which has been shown, based on a large dataset to be protective overall for a large dataset. This would lead to a meaningful tier 1 earthworm risk assessment avoiding a large number of false positives and minimizing the risk of producing false negatives.

99
A tiered Collembola risk assessment approach under the EU Regulation 1107/2009
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According to the EU Regulation 1107/2009 the toxicity of a plant protection product (PPP) for Collembola is assessed with the standard Collembola reproduction test (OECD 232, *F. candida*). If a potential risk cannot be excluded in the tier 1 risk assessment by comparing no effect level in the OECD test (including an assessment factor) and the predicted environmental concentration in soil (PEC_{soil}) higher tier studies are suggested, i.e. semi-field studies or field studies under realistic use conditions. However, the PEC_{soil} modeling procedure is about being revised in the near future by EFSA. PEC_{soil} are estimated to increase by a factor of up to 40 leading to a strong increase in Tier 1 risk assessment conservatism. In order to buffer an unreasonable increasing number of false positives in an overly conservative tier 1 risk assessment intermediate tiered alternatives are needed. Therefore, a toolbox for a tiered Collembola risk assessment approach is discussed: 1) The standard Collembola reproduction test with *F. candida* (OECD 232) where the PPP is mixed homogenously into artificial soil is a suitable tool to detect the intrinsic toxicity of a PPP to Collembola in a first step. 2) To account for a more realistic exposure in an ´extended` laboratory test system, the PPP can be applied e.g. via treated seeds or granules in the OECD 232 test. 3) A Collembolan two-generation study is developed as an intermediate tiered laboratory test system in order to assess the intrinsic potential for recovery from the treated population itself. 4) In semi-field test systems, e.g. Terrestrial Model Ecosystems (TME), the short- and long-term impact of a PPP on Collembola communities can be tested in soil cores. Comparative measurements with field data indicate that a TME is an intermediate tiered test system. 5) A Collembola field study under realistic use conditions is suggested as highest tier within the tiered Collembola risk assessment approach, including typical crop specific agricultural management practices. Case studies will be presented and demonstrate that the proposed intermediate tiered test systems are conservative and fit well into the tiered risk assessment scheme.

100
Supporting Agrochemical Registration Through Bespoke Multi-Country Data Collection
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Current regulations governing the registration of plant protection products in the European Union (1107/2009) mandate that an assessment for the potential to reach groundwater is made. This is primarily undertaken using conservative modeling approaches. Registration of plant protection products is becoming increasingly difficult within the EU as the risk assessment framework has evolved to make the parameterization of these models more conservative. At present, however, there is no generally accepted higher tier option that can be used to put conservative modeling estimates in to context. Monitoring presents a potential higher-tier option to demonstrate zero or very low exceedence of groundwater concentration limits when modeling suggest the potential for extensive movement. To support the registration of a pesticide and address EU and member state questions, a very large pan-European groundwater testing programme has been designed and undertaken to establish if this product has the potential to leach to groundwater following commercial use. The methodology of the study will be outlined, alongside how key challenges have been overcome, and initial findings of the study will provided.

101
Fate of rice herbicide Propanil and its metabolite 3, 4-Dichloroaniline in water-sediment systems from rice paddy with and without flooding simulation
Y. Yuan, P. Zhang, C. Possberg, RWTH Aachen University / Institute for Environmental Research Biology V; M. Romich, RWTH Aachen University / Institute of Sociology; B. Scholz-Starke, RWTH Aachen University Institute for Environmental Research / Institute for Environmental Research BioV; H. Hollert, RWTH Aachen University / Institute for Environmental Research; M. Ross-Nickoll, RWTH Aachen / Institute for Environmental Research; A. Schaeffer, RWTH Aachen University / Institute for Environmental Research
Fate of rice herbicide Propanil and its metabolite 3, 4-Dichloroaniline in water-sediment systems from rice paddy with and without flooding simulation Ye Yuan[1], Peng Zhang[1], Burkhard Schmidt[1], Manfred Romich[2], Bjoern Scholz-Starke[1], Henner Hollert[1], Martina Ross-Nickoll[1], Andreas Schaeffer[1]
[1]Institute for Environmental Research (Biology V), RWTH Aachen University, Aachen, Germany [2]Institute of Sociology, RWTH Aachen University, Eilfschornsteinstr 7, 52062 Aachen, Germany E-mail contact: ye.yuan@bio5.rwth-aachen.de In order to determine the fate and behavior of a model pollutant in Yangtze water sediment systems, we selected the rice herbicide Propanil, which is known to release the priority pollutant 3,4-dichloroaniline (DCA). DCA, which could also be formed by many other pesticides and has been reported to form a toxic metabolite in soil and sediment, i.e., tetrachloro-diazobenzene (TCAB), which we included in our fate studies. We incubated Propanil in water sediment collected from rice agriculturally influenced area of the Three Gorges Reservoir area at Yangtze during dry season and simulated flooding conditions to the system in lab, applying radioactively labeled compounds in order to establish mass balances and to figure out further metabolisms as well. The fate of ^{14}C labeled DCA was also studied similarly in a water-sediment system collected from a rice paddy area in Italy. Besides, qualitative interviews with farmers and pesticide shop owners/ retailers /wholesalers were also carried out in order to figure out the influencing factors of farmers' choices on pesticides/herbicides related products, as well as to know farmer's pesticide usage habits, annual agricultural activities, related knowledge and environmental awareness, and so on, which is a supplementary support for the environmental risk assessment of pesticide use in this area.

102
Environmental quality through science? – Reflecting "the new challenges" for pesticide risk assessment
T. Frische, Federal Environment Agency UBA / Section IV
In March 2013 the three independent European Scientific Committees (SCHER/SCENIHR/SCCS) adopted their opinion "Addressing the New Challenges for Risk Assessment". The opinion was delivered upon request of the European Commission asking for "a comprehensive review of risk assessment procedures and new challenges for risk assessment". Regarding ecological risk assessment of organic chemicals, the opinion concludes: "The approaches in current use for ecological risk assessment are likely to suffice for regulatory purposes as sufficiently protective for ecosystems. However they lack environmental realism. This entails high uncertainty on the actual consequences of environmental contaminations on the ecosystem structure and functions that has to be addressed by the application of uncertainty/safety/default factors. The main challenge for ecological risk assessment is to develop tools that take account of the complexity of the potentially exposed ecosystems and enable assessment of site-specific effects." Several priority areas for improvement are identified by the Committees in view of their "vision of future risk assessment methodology". Focusing on the state-of-the-art in pesticide risk assessment in Europe, the presentation is reflecting the SCHER/SCENIHR/SCCS-opinion against the following questions: What are the basic assumptions of the opinion? What is the evidence in support of these assumptions and the main conclusions of the opinion? What are the mechanism driving the evolution of risk assessment methodology? What are the roles of the main stakeholders (academic science, industry, government) involved in this process? What might be the consequences of the changes envisaged by the opinion (i.e. moving risk assessment methodology further towards increasing complexity)?

Plants and pollutants in the environment (II)

103
Strategies for enhancing plant survival and remediation potential under metal toxicity
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The natural nutrient mining ability of plants has been widely explored in the quest for evolving sustainable strategies for remediating toxic contaminants in the environment. Some plants have been discovered to possess inherent ability to accumulate high amounts of contaminants and referred to as hyperaccumulators. Apart from simply classifying plants as either phytoremediator or as non-phytoremediator, the mechanism(s) behind the phenomenon remain poorly understood. Moreover, despite the unique contaminant accumulation potentials of these phytoremediatiors, they often suffer from the toxic effects of the contaminants they are to remediate, and remediation efficiency remains poor. We explored the survival and remediation potential of *Brassica juncea* plants (a well-known phytoremediatior) under soil contaminated with zinc sulfate and compared it to that of *Vicia sativa* (a leguminous plant). *B. juncea* plants exposed to 600 mg kg^{-1} Zn over 8 weeks exhibited poor Zn growth with a tolerance index of 5.0% while *V. sativa* under similar conditions significantly survived Zn toxicity better with a tolerance index of 82.0%. A synchrotron based m-XAFS analysis of the plants revealed that *V. sativa* stores Zn in its root in the chelated forms of Zn histidine, Zn carbonates and Zn phytate whereas the roots of *B. juncea* plants were dominated by toxic Zn sulfate and Zn oxalate. Despite the significantly high tolerance index of *V.sativa*, it exhibits poor remediation potential (1.5% Zn removal) compared to the low tolerant *B. juncea* plants (16.3% Zn removal). The possibility of *V.sativa* to confer its tolerance to the better remediating *B. juncea* was investigated under a mixed planting system. This significantly improved the tolerance index of *B. juncea* plants to 72.5% and also enhanced Zn removal to 33.0%. It was hypothesized that the Rhizobia bacterium symbiotic to the leguminous *V. sativa* was responsible for the better plant growth and remediation of the *B. juncea* plant. Evaluation of *B. juncea* plants inoculated with *Rhizobium leguminosarum* supported this hypothesis and the bacteria significantly improved plant tolerance and phytoremediation potential with m-XAFS analysis of plant roots revealing root storage of Zn as Zn Polygalacturonate, Zn phytate and Zn cycteine. This work therefore suggests bacteria induced changes in Zn speciation in plant is a key mechanism for plant survival under metal toxicity.

104
Modelling air-vegetation distribution of BaP in the Iberian Peninsula using biomonitoring data
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In this study, an extensive database on levels of atmospheric PAHs in pine needles from biomonitoring schemes in the Iberian Peninsula fuelled the establishment of the first models of behaviour for PAHs, using the example of benzo[α]pyrene (BaP). The modelling system WRF+CHIMERE was implemented with high spatial and temporal resolution to the Iberian Peninsula (9 km, 1 hour), using BaP atmospheric levels collected over a year-long sampling scheme comprising 4 campaigns (one per season) in over 30 sites in Portugal and a single sampling campaign in the NE of Spain, also in over 30 sites. Information on meteorological parameters was collected from the weather stations closest to the sampling sites. Climatology of the levels and transport patterns (e.g. dispersion) established for air by the model and the estimated values in vegetation were validated with the concentrations in pine needles, whereas the estimated levels in air from the values in pine needles were obtained by 5 different approaches. The justification for this study is the gaps still existing in the awareness of the life cycles of SVOCs, particularly the partition processes between air and vegetation. The comparison of the levels on a regional scale will enable the strong enhancement of the knowledge available in the current scientific literature for studies of atmospheric chemistry and transport of trans-boundary pollutants, which is scarce (and even more if we consider its model validation against experimental data). The WRF+CHIMERE modeling system accurately reproduces the spatio-temporal patterns of concentrations of BaP over the vegetation in the Iberian Peninsula (errors lower than 50% for all stations and seasons). Several methods have been tested to convert vegetation concentration (pine needles) to atmospheric concentrations; therefore they have been used as biomonitors of BaP over the IP. The results indicate that, from the several methods tested, the St. Amand approach reveals the most accurate results when compared both to modeling results and observations from the EMEP data network. Acknowledgements: This work has been partially funded by the European Union Seventh Framework Programme-Marie Curie COFUND (FP7/2007-2013) under UMU Incoming Mobility Programme ACTion (U-IMPACT) Grant Agreement 267143.

105
Bioaccumulation of ionized and neutral emerging contaminants: Exposure of edible crops via solid and liquid effluent streams
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Many wastewater- derived anthropogenic organic contaminants persist through treatment processes either dissolved in the aqueous stream or sequestered by sorption to sludge solids. These contaminants of emerging concern (CECs) represent a diverse chemical suite and include but are not limited to pharmaceuticals, flame retardants, corrosion inhibitors, surfactants, and plasticizers. Concerns arise when reclaimed water becomes a viable resource for agricultural irrigation, or when wasted sewage sludge solids (biosolids) are applied to agricultural lands as a soil amendment. This study examined strawberry and lettuce plants grown under greenhouse conditions with reclaimed water fortified at eight levels of increasing contaminant doses. The accumulation in the edible portion was found to correlate with dose for many of the selected target contaminants in lettuce leaf tissues, while the strawberry was found to accumulate two chlorinated organophosphate flame retardants (TCEP, TCPP) in a dose-dependent manner. Further investigation involved the examination of CEC accumulation in other tissues of the plant including roots (both crops) and stems/leaves (strawberry). Root concentration factor (RCF) and translocation factor (TF) values were calculated to improve understanding and possible predictive capability of how CECs will be transported and accumulated in plant tissues that may be consumed. Additionally, lettuce crops were grown in control and biosolids-amended soils, and the edible leaf portions examined for uptake of CECs from the solid phase. Results from this study have important implications with respect to the potential exposure of humans to contaminants in fresh produce. The ultimate goal of this ongoing project is to improve mechanistic understanding of plant uptake of CECs from both reclaimed water and amended soil pathways, thereby allowing for advancement of models intended to predict human exposure.

106
Phytoxicity of Azoles from Personal Care Products and Pharmaceuticals in the Absence and Presence of Biosolids
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Sewage sludge may be a valuable agricultural fertiliser. However, it also represents

a sink for poorly degradable and highly adsorptive wastewater-born pollutants. One group of agents frequently detected in sewage sludge are fungicides from the structural group of azoles. They are applied in plant protection products but also in cosmetics and pharmaceuticals. Climbazole, ketoconazole and fluconazole are contained in anti-dandruff shampoos, systemic antimycotics and skin creams, respectively, and have been measured in sewage sludge at concentrations between 50 and 4450 μg/kg. Despite their high efficacy against fungi and the recent indication for specific toxicity toward plants, their possible impact on the terrestrial environment has so far only poorly been studied. The aim of this work was therefore to characterise their phytotoxicity and its dependance on biosolids amendment since the environmental exposure route will be through sewage sludge application. To this end, plant growth inhibition tests were conducted according to the OECD guideline 208 using *Brassica napus* with and without addition of biosolids to the test soil at a realistic application rate of 3.8 g biosolids d.w./kg soil d.w.. For fluconazole, the lowest EC_{50} was determined with 9.3 mg/kg soil d.w. for shoot biomass. The presence of biosolids had no impact on its toxicity when comparing the effective concentrations with and without biosolid amendment. This is in agreement with the expectation because fluconazole is water soluble and ionic at environmentally relevant soil pH and thus not likely to be bound by sewage sludge. First results for climbazole and ketoconazole indicate that they are less phytotoxic than fluconazole with an EC_{50} of 30 mg/kg soil d.w. and 666 mg/kg soil d.w., respectively, in the absence of biosolids. We assume that in contrast to fluconazole, for climbazole and ketoconazole the bioavailability, and hence the phytotoxicity, will be reduced in the presence of biosolids.

107
Impact of deposition produced during the static testing of solid rocket motors on corn and alfalfa
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Tests of horizontally restrained rocket motors at the ATK facility in Promontory, Utah, USA, result in the deposition of an estimated 1.5 million kg of entrained soil and combustion products (mainly aluminum oxide, gaseous hydrogen chloride and water) on the surrounding area. The deposition is referred to as test fire soil (TFS). Farmers observing TFS deposited on their crops expressed concerns regarding the impact of this material. To address these concerns, we exposed corn and alfalfa to TFS collected during a September 2009 test. The impact was evaluated by comparing the growth and tissue composition of controls relative to the treatments. Exposure to TFS, containing elevated levels of chloride (1000 times) and aluminum (2 times) relative to native soils, affected the germination, growth, and tissue concentrations of various elements, depending on the type and level of exposure. Germination was inhibited by high concentrations of TFS in soil, but the impact was reduced if the TFS was pre-leached with water. Biomass production was reduced in the TFS amended soils and corn grown in TFS soils amended did not develop kernels. Chloride concentrations in corn and alfalfa grown in TFS amended soils were two orders of magnitude greater than controls. TFS exposed plants contained higher concentrations of several cations, although the concentrations were well below livestock feed recommendations. Foliar applications of TFS had no impact on biomass but some differences in the elemental composition of leaves relative to controls were observed. Washing the TFS off the leaves lessened the impact. Results indicate that the TFS deposition could have an effect, depending on the amount and growth stage of the crops, but the impact could be mitigated with rainfall or the application of additional irrigation water. The high level of chloride associated with the TFS is the main cause of the observed impacts.

108
Genotoxicity of Contaminated Drinking Water Sources Detected by a Plant Bioassay
O.P. Olorunfemi, University of Benin / Animal and Environmental Biology; M.I. Chigozie, University of Benin / Department of Plant Biology Biotechnology; D.I. OLORUNFEMI, University of Benin / Plant Biology Biotechnology
Climatic conditions are important factors that influence the level of contaminants present in water sources and the extent of their toxic effects on plant and animal life. In this study, water samples from a borehole and a man-made lake used for domestic and human consumption in Ozomu, a rural community in Edo State, Nigeria were obtained in the dry and wet seasons and subjected to macroscopic and microscopic evaluations using the *Allium cepa* root test. Compared to the control (tap water), all the water samples induced significant ($p< 0.05$) inhibition of root growth and increased frequency of chromosomal abnormalities in a season-dependent relationship. Our monitoring approach with the plant bioassay was able to detect contamination related to the drinking water sources. In particular, the results indicate that seasonal variation has influence on the level of toxic and genotoxic contaminants in the lake water which may be of health risk to the inhabitants who use it without prior proper purification treatments. *Key words: Allium cepa*, genotoxicity, lake water, public health.

REACH after the second registration deadline:

Environmental challenges (II)

109
Data availability, data requirements and opportunities for improvements in the hazard assessment under REACH
M. Scheringer, ETH Zuerich / Institute for Chemical and Bioengineering

110
Systematic analysis of REACH data and their use in screening and prioritisation
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ECHA has so far received approximately 38 500 new registrations under REACH (excluding notifications to Member State Competent Authorities under the previous European chemicals legislation - Directive 67/548/EEC), out of which 31 000 full registrations, covering approximately 7500 distinct substances. For 5000 of these substances not registered exclusively as an isolated-intermediate, industry has submitted one or more technical dossier with one or more study summary for each of the REACH endpoints required for the corresponding tonnage band according to the REACH Regulation. Overall, ECHA has received over 900 000 endpoint study records and approximately 15 000 chemical safety reports. At the same time, the Classification and Labelling Inventory (C&L) has been populated with more than 3 million notifications for over 120 000 substances according to the requirements of the CLP Regulation. These submitted dossiers and notifications are the most important sources of information for carrying out the activities of all REACH and CLP processes, including Compliance Check, Substance Evaluation and Risk Management. The large amount and complexity of information in ECHA's registration database and the C&L Inventory presents opportunities for a more effective regulation of chemicals but also a number of challenges. In addition to disseminating these data in a comprehensible and useful form to the general public, Member State Authorities and ECHA need to use the data for verifying safe use and impose risk management measures when needed. At the same time, it is necessary to ensure high quality of the standard information as required by the REACH Regulation, particularly when the submission of hazard and exposure data that do not meet the standard information requirements can lead to different hazard and risk conclusions. The REACH processes to increase dossier quality and identify substances that require risk management are clearly distinct. However, they allow the use of similar methods and thus provide opportunities for synchronisation and coordination for maximum efficiency. Given these challenges, ECHA has made significant steps towards developing methodologies and tools to allow the systematic analysis of registration and notification data. This presentation will describe the underlying machinery behind these tools and will exemplify their recent applications in screening and prioritisation activities in ECHA.

111
Scientific options for reducing acute and chronic fish toxicity tests in the regulatory assessment
A. Kienzler, European Commission Joint Research Centre

112
Future scientific challenges faced in Environmental Risk assessment
J.V. Tarazona, European Food Safety Authority / Pesticides Unit

113
R&D needs and knowledge gaps
D. KNIGHT, ECHA
ECHA administers the REACH, CLP and Biocidal Products Regulations, and therefore we have a good understanding of the limitations of current regulatory science and where there are needs for fundamental scientific research and applied R&D. The research we suggest will also be useful in other regulatory schemes covering chemicals and chemical products. In addition to long-term fundamental research, we suggest R&D leading to results of practical use in the medium term, e.g. to be of benefit for 2018 REACH registration. R&D should as far as possible build on and incorporate current methods, with targeted development when appropriate, to produce 'toolkits' with methodologies to combine the data into an overall prediction (e.g. as IATAs). The aim would be to produce generally-accepted and scientifically-valid, 'fit for purpose' techniques that are fairly standardised yet still flexible. To support such combined approaches further fundamental research will be needed into the biological mechanisms that underpin (eco)toxicity effects (e.g. AOPs). There are two needs for assessing the hazardous properties of chemical substances: To screen a large set of substances to select groups with particular characteristics for further action. To assess a specific substance for a defined purpose, e.g. to fill a 'data gap' and establish safe use from a risk assessment.

Therefore combined-approach assessment techniques should be fit for the purpose the prediction is to be used for and also flexible enough to allow substance specific adjustments. The degree of uncertainty tolerated in the prediction depends on the regulatory purpose: therefore assessing and communicating uncertainty is a key element. In general, higher certainty is needed to assess specific individual substances than for screening sets of chemicals for priority setting. Regulators generally set standards for the information on the properties of chemicals, whether from standard tests or non-standard approaches; hence if the prediction does not meet the standard it is not fit for purpose.

114
Discussion and wrap-up
A. Kapanen, European Chemicals Agency ECHA

Ecotoxicology in tropical and polar regions (III)

115
An Antarctic research station as a local source of perfluorinated organic pollutants
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Persistent organic pollutants (POPs) are industrial chemicals and ubiquitous global contaminants. With an increasing number of chemicals fulfilling the classification criteria of POPs, and increasing human activity in Polar Regions, the potential for direct introduction of pollutants to high latitude environments is enhanced. Many consumer products, including textiles and furnishings contain perfluorinated compounds (PFCs), including PFOS recently annexed under the Stockholm Convention. As people visit and work in Antarctica these products are just as likely to be used there as they would be in other parts of the world. Although highly stable, these compounds can leach out of products and be released into the surrounding environment. In this study we investigate Australia's Casey Station as a local source of PFCs to the environment and evaluate accumulation in various biological matrices as evidence of human activity introducing recently listed POPs directly into the Antarctic environment. Samples of five different abiotic and biotic matrices were collected from Casey Station and its immediate marine and terrestrial environment, namely; indoor dust, secondary treated wastewater, lichen, moss, and marine amphipods. Of the 16 PFCs analysed for, 15 were detected in the indoor dust collected. PFOS was detected at the highest concentration, more than 10 times higher than any other compound. PFOA was also found at notable concentrations. The levels of PFOS and PFOA detected in this study mirror the levels typically seen in houses and other buildings from Australia and other developed countries. More recently constructed parts of the station had lower levels of PFOS than older areas perhaps indicating a decrease in usage of PFOS since its restriction. Secondary treated wastewater samples were dominated, in both the dissolved and particulate phases, by PFOS, with PFOA and PFDS also present. The concentrations of PFCs were 2 – 6 times higher in the dissolved phase than in the particulate phase. The levels of PFCs found in wastewater from Casey Station are similar to those seen in treated wastewater from Australia and other developed countries. Only negligible or below detection limit concentrations of PFCs were detected in moss, lichen and amphipod samples. These results indicate that Antarctic research stations such as Casey are acting as local sources of PFCs into the Antarctic environment, increasing the continents level of pollution.

116
Mobilisation of Persistent Organochlorine Burdens in Migrating Southern Hemisphere Humpback Whales
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The dependence of polar species on lipid rich diets to sustain the temperature and productivity extremes of high latitude environments, makes them particularly susceptible to bioaccumulation of lipophilic persistent organic pollutants. Marine mammals are at the greatest risk of accumulating toxic levels of these chemicals due to their trophic level, longevity and high proportion of body fat. Southern hemisphere humpback whales (*Megaptera novaeangliae*), feed in the Southern Ocean and overwinter in tropical breeding grounds, seasonally undergoing the longest migration and fasting events known in any mammal. The extreme life history behavior of these populations provide a unique opportunity to study the toxicokinetics of POPs during a period of chronic energy deficit. Medical research has previously evidenced the toxic effects associated with rapid weight loss and concomitant mobilization of POP burdens. This study targeted free-swimming males humpback whales at two time points on their annual migration along the east coast of Australia (breeding stock E1). Biopsies were collected from 58 individuals and analysis of superficial blubber yielded a comprehensive overview of contaminant accumulation in Southern Ocean foraging baleen whales. Further, significant differences between northward migrating (early migration, post-summer feeding) and southward migrating (late migration; fasted) cohorts was observed. The concentration indece (CI), defined as the average compound concentration in southward versus northward migrating cohorts, were consistently greater than 1. The average CI of compounds detected in ≤50% of both early and late cohort animals was 5.1. The results of this study emphasize the importance of considering prolonged periods of food deprivation when assessing chemical risks posed to wildlife. This is of particular importance for Polar biota adapted to extremes in ecosystem productivity.

117
Persistent organic pollutants and trace elements in blood of the wandering albatross: influence of individual traits and foraging ecology
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Marine Antarctic and subantarctic environments are contaminated by persistent organic pollutants (POPs) and trace elements through atmospheric transport and wet and dry deposition processes. Seabirds are regarded as cost-effective bioindicators of environmental contamination, because they integrate biomagnifying pollutants *via* food intake. Several intrinsic and extrinsic factors may drive intra-specific variation in seabird contamination, but this has rarely been studied. Here we measured POPs (10 organochlorine pesticides, OCPs, and 7 polychlorinated biphenyls, PCBs) and trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V and Zn) in blood of 169 individuals of wandering albatross *Diomedea exulans*, a long-lived (50+ years) and wide-ranging top predator, sampled at the Crozet Islands, southern Indian Ocean. Thanks to an exceptional long-term dataset, the effect of individual traits (sex, age and breeding status) and foraging ecology on contamination could be assessed. Wandering albatrosses carried remarkably high loads of contaminants in their blood, with strong between-individual differences. In several cases, contamination differed between sexes, which could be due to sexual-specific physiological dynamics of contaminants. For instance, females had lower levels of OCPs, as they can excrete organic lipophilic pollutants into eggs. Breeding status affected blood levels of some contaminants. For example non-breeding birds had lower Hg levels than breeding ones. Age was not expected to influence contaminant levels, since blood reflects recent exposure (i.e. days to several weeks), rather than accumulation through time. Yet, blood Hg levels decreased with age in males, but not in females. As aging male wandering albatrosses were shown to change their foraging habitats, old individuals could be exposed to lower Hg levels through their diet. Feeding habitat (inferred from carbon stable isotope ratios) had indeed a strong effect on blood Hg levels, with individuals feeding in warmer subtropical waters being more contaminated than those feeding in colder subantarctic waters. The opposite relationship was shown for the sum of OCPs, potentially translating their enhanced deposition at higher latitudes. In conclusion, this work provides new insights into the contribution of different intrinsic and extrinsic factors on intra-specific variations of contaminant levels in seabirds, stressing the critical role of individual foraging specialisation.

118
Temporal trends of persistent organic pollutants in arctic foxes from Svalbard in the light of a changing climate
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The present study investigates concentrations and temporal trends of persistent organic pollutants (POPs) in arctic foxes (*Vulpes lagopus*) from Svalbard, Norway, considering changes in food availability and feeding habits. We analysed liver of 100 arctic foxes, collected in 1997/98, 1998/99, 1999/00, 2001/02, 2002/03, 2003/04 and 2010/11 for concentrations of polychlorinated biphenyls (PCBs; PCB -28, -52, -101, -118, -138, -153 and -180), chlordanes (*cis*-chlordane, trans-nonachlor and oxychlordane), *p-p'*-DDT, *p-p'*-DDE, HCB, mirex and β-HCH. Number of reindeer carcasses in Adventdalen and sea ice coverage of Isfjorden in the spring preceding the trapping season were used as indexes for climate influenced food availability between years. Stable isotope analyses were performed to assess feeding habits in the last months before trapping. The POPs found in highest concentrations were oxychlordane, PCB-180 and PCB-153. Concentrations of ∑PCBs (PCB -118, -138, -153, -180) and ∑chlordanes (trans-nonachlor and oxychlordane) decreased over time when controlling for possible confounding variables. We also found evidence for an effect of body condition and $\delta^{13}C$ on the POP concentrations, as thinner foxes and foxes feeding from the marine food web had significantly higher levels of POPs. There was no effect of reindeer mortality and sea ice coverage on the concentrations of POPs, although increased reindeer mortality had a non-significant negative effect on all

the POPs analysed.

119

The endocrine disruption properties of an adipose contaminant mixture extracted from East Greenland polar bears studied in the H295R cell line

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In recent years there has been much debate on the potential risk of chemicals that have been classified as endocrine disruption compounds (EDCs). Human and environmental risk assessment of EDCs is a challenge since the exposure to humans and wildlife is a complex mixture of many known and unknown compounds, where some can be in the parts-per-billion (ppb) concentration range. However, most reported studies on endocrine disruption effects have been on single compounds at concentrations higher than environmentally relevant. The presence of persistent organic pollutants (POPs) in arctic wildlife has been well described and especially the polar bear (*Ursus maritimus*) is recognized as being one of the most contaminated species in the Arctic. The present study investigated the *in vitro* endocrine disruptive effects of the POP mixture found in adipose tissue from 10 East Greenland polar bears collected in 2011. Specifically, the tissue extracts were tested for steroidogenic activity in the human adrenocortical carcinoma cell line H295R. These extracts contained a complex mixture of measured polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and brominated flame retardants (BFRs). GC-MS/MS analysis showed that all 10 adipose extracts caused significant changes to the hormone levels in H295R compared to solvent controls. Levels of progestagens expressed upstream of the enzyme CYP17 (pregnenolone and progesterone) increased with a corresponding decrease in androgens expressed downstream (dehydroepiandrosterone and androstenedione). These results demonstrated comprehensive *in vitro* effects of POPs extracted from polar bear adipose tissue on key elements in the steroidogenesis, and identifies disruption of CYP17 activity as a mode of action. A POP-induced interference with CYP17 can potentially explain previously observed hormone levels in polar bears and could pose a risk to their reproductive health.

120

Mercury and persistent organic pollutants as endocrine disruptors in polar seabirds

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Added to climate change, Polar Regions are also subjected to high levels of environmental pollutants such as persistent organic pollutants (POPs: PCBs and pesticides) and heavy metals, as mercury. This additional input in the environment represents a supplementary challenge for top predators such as seabirds which accumulate high levels of these contaminants. However the precise impact of mercury and POPs as endocrine disruptors has been poorly investigated in free-living organisms. Using field studies on 2 polar seabirds (Black-legged kittiwakes in the Arctic and snow petrels in Antarctica) we investigate the specific roles of mercury and POPs on several major endocrine mechanisms (Luteinizing hormone and prolactin, 2 key pituitary hormones for the onset of reproduction and parental behavior; and corticosterone, the stress hormone, which mediate major reproductive decisions). In both species, increasing levels of mercury were clearly related to decreasing levels of Luteinizing hormone and to a greater probability of non-breeding. In snow petrels, high blood mercury levels were associated with low prolactin secretion and most contaminated individuals were less committed into incubation behavior. Corticosterone secretion was clearly affected by POPs in both species: the most polluted birds had a stronger stress response and would maintain high levels of corticosterone post-stress. Those findings underline two different pathways used by pollutants to impair breeding, as mercury would rather affect pituitary hormones and POPs the hypothalamo–pituitary–adrenal (HPA) axis. We will then discuss the possible mechanisms disrupted and the potential long-term demographic impacts of contaminant exposure in polar seabirds.

Marine and coastal ecotoxicology and risk assessment (III)

121

Harmful Algal Blooms and associated toxins: mussel larvae at risk?

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Due to the combined effect of overfishing, climate change, eutrophication and the dispersal of invasive species in the marine environment, harmful algal blooms (HABs) are steadily increasing in frequency, intensity and geographical scale. Yet to date, our understanding of the effects of HABs on the recruitment of marine organisms is limited. As shellfish farming is expected to play a crucial role in the fulfilment of the future global protein demand, a better understanding of the risks that HABs pose to the reproduction and development of bivalves is needed. This research therefore aimed to investigate the acute toxic effects of harmful algae and marine toxins on the larval viability, development and innate immune response of bivalves. To this end, embryos of the model species *Mytilus edulis* (blue mussel) were exposed for 48 hours to a concentration series of the marine toxins domoic acid (DA) and okadaic acid (OA) as well as their respective toxin-producing algae *Pseudo-nitzschia multiseries* and *Prorocentrum lima*. We found that neither bloom concentrations of domoic acid, nor bloom densities of *P. multiseries* displayed acute toxic effects on the viability and development of *M. edulis* larvae. Okadaic acid on the other hand significantly reduced the viability of veliger larvae at concentrations as low as 37.8 $\mu g.l^{-1}$, which is a concentration range likely to occur during dense blooms of OA producing algae. This effect may be related to a significant inhibition of larval protein phosphatases by OA as observed *in vitro*. *P. lima* was not found to affect the larval development or viability but induced an increase in the phenoloxidase innate immune activity which could not be attributed to the occurence of OA in the algae. A similar increase in PO activity was detected for *P. multiseries*. This effect was strain dependent and could partially be attributed to the presence of DA. As this is the first study to investigate and detect the activation of the phenoloxidase innate immune activity by harmful algae and marine toxins, the consequences of these increases for the larval resilience to other stressors are unknown. Yet the change of the immune activity combined with the reduced viability associated with OA warrants closer investigation as HABs could possibly influence natural recruitment as well as the hatchery-cultured production of bivalves.

122

The use of eco-toxicological tests for characterization of contaminated soil from boat activities

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The object of this study was to study a boat maintenance facility by investigating the degree of contamination and assessing how leachate water from soil affects organisms from three trophic levels. Surface and subsurface (20 cm depth) soil samples were collected in a typical boatyard (200 boats, 12000 m^2) at a 70 m (station A), 90 m (station B), 120 m (station C), and 160 m (station D) distance from the shoreline. Three replicate samples, ca 10 m apart, were taken at stations A, B and C, respectively, and one replicate was taken at station D, (i.e. altogether 20 samples with 10 at surface and subsurface, respectively. The total copper (Cu), lead (Pb), tin (Sn) and zinc (Zn) concentrations were determined for all replicates. Pooled samples from the respective stations were used for analysis of organotin compounds, irgarol and polyaromatic hydrocarbons. Leachate waters were produced from the pooled samples and used for toxicity testing with the bacterium *Vibrio fischeri*, the macroalga *Ceramium tenuicorne,* the macrophyte *Myriophyllum spicatum* and the crustacean *Nitocra spinipes*. Very high concentrations of Cu, Pb, Zn were detected, with maximum values of 16 300, 6 430 and 18 600 mg/kg dw, respectively. Organic hazardous compounds were found in high concentrations with maximum values of 37, 27 and 16 mg/kg dw for tributytin (TBT), dibutyltin (DBT) and triphenyltin (TPhT), respectively. All pollutants exceeded existing guidance values for both sensitive land use and less sensitive land use by several factors, in both surface and subsurface soil. The least and worst cases of total amount of TBT (12 000 m^2 and 0.2 m depth) were estimated to be 10 and 122 kg of TBT. Leachates were shown to be toxic in all three test organisms. Several known hazardous pollutants were found in boatyard maintenance areas and they exceeded recommended guidance values by several factors. Leachates were shown to be toxic to test organisms of several trophic orders. This underlines that boat maintenance facilities in general should be better regulated to minimize further exposure to humans and spread of contaminants in the environment. The amounts of contaminants accumulated in these areas calls for investigations of how remediation should be performed.

123

Physiological responses of Mytilus galloprovincialis under multiple stressors: nutritive stress and PAHs

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Large spatial scale mussel monitoring programs are characterized by a great variability of environmental conditions which cause important differences in the physiology of the animals that inhabit them. One of main differences encountered is the trophic condition which is highly variable when different habitats are

monitored. Moreover, a low availability or even the total absence of food during variable periods is a normal feature in marine ecosystems. Mussels, such as *Mytilus galloprovincialis*, are extensively used as sentinel species of pollution in marine coastal monitoring programs. Natural food availability in the studies areas could be inferred from mussel condition index, whose high variability has been repeatedly evidenced in the N-NW Spanish Marine Pollution Monitoring Program [1][2]. From such studies, it has been stated that biological responses caused by pollutants seem to be masked by biological variables as mussel condition. In this context, a laboratory experiment was designed to determine the relative importance of two stressors: low food availability and the presence of toxicants on some physiological biomarkers. Two experimental trophic conditions were simulated by regulating daily food ration: low and high food availability which promote negative and positive mussel energy balances, respectively. In both cases, mussels were exposed to a concentration range of the polycyclic aromatic hydrocarbon (PAH) fluoranthene (FLU).

124

Health status of cod (Gadus morhua) at dumpsites for chemical warfare agents in the Baltic Sea

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After World War II, large amounts of chemical weapons stored on German territory were dumped in the Baltic Sea by order of the allied forces . In addition of being a cheap method of disposal, the belief was that the vast amounts of waters in the oceans would neutralize and absorb the dangerous substances. At least 40.000 tonnes of chemical munitions containing an estimated 13.000 tonnes of chemical warfare agents were dumped in the Baltic Sea, primarily in the Bornholm Basin. Other official dumping sites were the Little Belt area and the Gotland Deep. There is evidence, however, that also other areas besides the offical CWA dumpsides are contaminated by chemical warfare agents (CWA). CWA were dumped as artillery shells, aircraft bombs or in containers; partly entire ships loaded with munitions were sunk. Today, munitions are in different stages of decomposition. Metal shells are corroding and contents are leaking into the environment at a rate that has not been measured so far, posing a possible risk for the Baltic Sea ecosystem. In previous studies, several CWAs of major concern for biota, such as inorganic arsenic and organo-arsenic compounds, have been found in the sediments within and around dumpsites (Missiaen & Paka 2007). Unaware of this risk, human sea-bottom activities, such as bottom trawling, constructions of pipelines and cables as well as windfarms are increasingly claiming space within the contaminated areas. The aim of the present study was to increase knowledge on the bioavailability and biological effects of CWAs on fish, using a suite of biomarkers in an integrated approach.

125

Hazard and risk of herbicide contamination for marine microalgae: a field relevant approach

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Coastal waters are among the most productive ecosystems on the planet. Yet, they also receive a high contaminant load due to riverine inputs, land run-off and shipping activities, resulting in high concentrations of contaminants. Exposure of microalgal communities to these contaminants can potentially result in shifts of microalgal species which can, as they form the basis of marine food webs, ultimately affect the carrying capacity of marine and estuarine ecosystems. However, a comprehensive understanding of the actual hazard and risk of contaminants towards the microalgal community in coastal and estuarine waters is currently lacking. Therefore, a series of field relevant experiments was performed which included: 1. single species tests with different microalgal species and herbicides; 2. a multi-stress experiment with field relevant light conditions and 3. an outdoor multi-species experiment with a natural phytoplankton community. The hazard of the herbicides atrazine, Irgarol®1051, diuron and isoproturon was determined for three different microalgal species and a clear compound and species specific effect was demonstrated. Although the risk at current field concentrations was low, occasional harmful peak concentrations reaching potential effect levels do occur. Additionally, a risk for microalgae under the current environmental legislation (MAC-EQS) of several compounds was demonstrated. In a multi-stress experiment in which different seasonal light conditions were mimicked, herbicide toxicity was demonstrated to be significantly ($p< 0.05$) higher under spring light conditions compared to autumn light conditions. This observed season specific toxicity suggest that standard test conditions might under or overestimate the hazard of a compound in the field. In an outdoor multi-species experiment, the photosynthetic efficiency as well as the community composition of a natural phytoplankton community were significantly ($p< 0.05$) affected at the MAC-EQS concentration of the herbicide diuron. This study provides information on the hazard and risk of herbicide contamination under environmentally relevant conditions. It can be concluded that in coastal and estuarine areas, the presence of anthropogenic contaminants might result in changes in species composition in natural phytoplankton communities and therefore might be a threat to marine ecosystems.

Periphyton as bioindicator and community model – critical review, work in progress, future perspectives

126

Direct and indirect effects of triclosan on periphyton under grazing pressure

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The influence of grazing in the algal triclosan toxicity was evaluated in fluvial biofilm communities. Biofilms were exposed to grazers and triclosan individually, as well as to their interaction. As expected, grazers heavily influenced the biofilm community, reducing algal biomass and keeping the biofilm in a young state of development, which resulted in higher P-uptake capacity and less efficient defence towards oxidative stress. Although triclosan has been described as a bactericide, both algae and snails were clearly affected. Toxic effects of triclosan (22µg/L) on biofilms were noticeable at both structure and functional level. The diatom mortality increased and the community changed its composition, growth forms and richness. Moreover, the bactericide addition had negative consequences for biofilm function, which lost P-uptake and detoxification capacity. Snail mortality was also enhanced by triclosan addition. The presence of grazers modulated triclosan toxicity by enhancing the structural membrane damage provoked by grazers with their scraping mouthparts, resulting in a cascade of contaminant effects from producers to higher trophic levels.

127

Community diversity and 3D structure of periphyton exposed to silver nanoparticles and silver nitrate

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Periphyton provides essential ecosystem services by primary production and release of O_2. Its sensitivity to environmental conditions make it an important bioindicator and small-scale community model. It is a potential receiving compartment for engineered NP. The highest number of NP containing consumer products involve Ag NP due to their bactericidal properties. These have been attributed to both NP and Ag^+ released in aqueous media. Most toxicity studies are based on short-term exposure of single species. The possible chronic effects on complex communities such as periphyton are unknown. We thus used periphyton exposed to Ag as a community model to study changes in diversity and structure. Ag was applied as PVP-stabilized AgNP or as $AgNO_3$ over 18 d under controlled conditions, to understand effects dependent on the Ag species as well as on concentration and time. Periphyton was analysed regarding genetic diversity (Automated Ribosomal Intergenic Spacer Analysi (ARISA), Terminal Restriction Fragment Length Polymorphism), composition of extracellular polymeric substances (EPS), and 3D structure. These data were complemented with species/genera identification. Dissolved, total, and periphyton-associated Ag was measured at each sampling point. Algal diversity decreased in biofilms treated with 20 μg/L Ag over time. Preliminary results acquired by ARISA of diatoms show a decrease in diatom genetic diversity after 18 d of exposure to 20 µg/L Ag NP. EPS size distribution was not influenced by 18 d of exposure to different Ag species and concentrations. Biofilms exposed to 2 µg/L $AgNO_3$ or Ag NP had a significantly lower C/N ratio in the polymer fraction indicating a lower amount of polysaccharides and a higher amount of proteins. The relative concentration of low molecular weight acids was decreased in EPS from periphyton exposed to 20 µg/L Ag. The porosity of the biofilms decreased significantly in the presence of 20 µg/L $AgNO_3$. It remained at control levels in AgNP exposed biofilms. Biofilm roughness increased in $AgNO_3$ treated periphyton but was less heterogeneous in the presence of AgNP. Based on the preliminary evaluation of our data we conclude that both AgNP and $AgNO_3$ induce changes in periphyton relative to exposure time, concentration, Ag species, and the endpoint assessed. A range of characterization methods is thus necessary to capture effects caused by Ag. There seem to be Ag species dependent and independent effects.

128

Combined ionic and toxic stress induces community tolerance in periphyton

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Taxonomy-based approaches like diatom indices are not flexible enough to diagnose causes of ecological impairment. Approaches combining functional and structural assessments may support a better mechanistic understanding of stressor interactions in multiple stressed environments. In aquatic communities stressors exert a selection pressure, increasing community tolerance. A suitable approach for evidencing these processes is the "pollution-induced community tolerance" (PICT) concept. PICT can be detected in short-term tests by appropriate metabolic responses. The PICT concept was developed to determine the effects of toxicants to communities and for autotrophic communities photosynthesis inhibition was mainly used as an endpoint so far. For analyzing effects of different stressor qualities on community tolerance, further metabolic tests need to be developed. High ionic loads and herbicides often co-occur in aquatic systems. Rotter et al (2013) found joint effects of high ionic loads and the herbicide prometryn on periphyton tolerance to the herbicide. However, combined effects on salt tolerance were not quantified, yet. The aim of this study was the quantification of salt tolerance of freshwater periphyton and the determination of potential combined effects from salt and toxic stress on community tolerance. Therefore, we established a metabolic test to quantify salt tolerance by using the amino-acid proline as an indicator. This test was applied in a microcosm study using periphyton as a model community. In a factorial design two different concentrations of NaCl were used as a single stressor and were combined with prometryn. Over a growth period of 4 weeks community parameters such as photosynthesis and community tolerance were measured. Proline proved to be a suitable metabolic test for detecting induced salt tolerance in periphyton. Combined stress of prometryn and NaCl caused a higher tolerance of periphyton to the toxicant (analysed by inhibition of photosynthesis) than expected from the sum of single stressors. Salt tolerance of periphyton to combined stress of prometryn and NaCl (analyzed as induced proline) was clearly higher than tolerance from periphyton exposed to the toxic stressor but in the range of the salt stressed communities indicating no interaction of these stressors in terms of salt tolerance. These functional changes will be discussed in view of the structural changes observed in the diatom community.

129
Seasonal variability of natural river biofilm tolerance and diversity in an urban contamination gradient
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The aim of this study was to investigate the seasonal variability of the biological response of periphytic communities (river biofilms) chronically exposed to multi-metallic pressure. Biofilms were grown on immersed plastic membranes at three sites on the Seine river along an urban pollution gradient upstream (site 1) and downstream (sites 2 and 3) from Paris (France). They were collected in September 2011, March 2012, July 2012 and December 2012 after a 3 to 5 weeks colonization period. We measured biofilm tolerance to Cu, Ni, Pb and Zn, using a PICT (Pollution-Induced Community Tolerance) approach with a previously developed short-term toxicity test based on bacterial β-glucosidase activity (the tolerance of the heterotrophic community was investigated). In order to reliably compare metal tolerance among biofilms, the EC_{50} values derived from the toxicity tests were normalized by the corresponding total suspended solids concentration of the biofilm suspensions used for the test. Bacterial and eukaryotic diversity was also assessed on biofilm samples collected in summer (July 2012) and winter (December 2012) using high-throuput 16S and 18S DNA sequencing. Normalized EC_{50} values globally increased from site 1 to site 3 reflecting the metallic pollution gradient measured in the river water collected at the three sites and confirming a previous study on biofilm metal tolerance with biofilms collected at the same sites in 2009. Cu tolerance levels appeared to be positively correlated to water temperature but no clear seasonal tendency could be found for Ni, Pb and Zn tolerance levels. Partial Least Squares Regression (PLS) was performed on biological data (Y variables) and metallic contamination and physico-chemical data (X variables). Temperature appeared as one of the most important variables in the PLS regression. This study shows that chronic *in situ* exposure to environmental concentrations of metals has a significant impact on natural biofilms. Biofilm tolerance to metals reflects metallic exposure levels. Yet temperature appears as an important environmental variable shaping community structure and response to toxic exposure which shows that the sampling date is an important parameter to consider when using natural river biofilms to assess the impacts of urban pressure.

130
Metagenomic sequencing of periphyton: Taxonomic and functional insights into marine biofilm communities
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The majority of metagenomic studies have so far focused on the free-living microorganisms in the human gut, soil or in the aquatic environment. An important form of microbial life, *the biofilm*, has been considerably overlooked in the metagenomics literature as such; only five percent of the publicly available metagenome data in the mg-RAST server is associated with microbial biofilms. Periphyton communities are complex multispecies biofilms harbouring a great diversity of organisms attached to inorganic, organic, living or dead substrata. In this study, we used metagenomics to identify periphyton organisms in the coastal marine environment of western Sweden, and assessed the major molecular mechanisms that predispose these communities for an attached life-style. We identified highly abundant phototrophic groups including macro- and micro-algae as well as *Cyanobacteria* and *Roseobacter*. Heterotrophic organisms are found to be dominating periphyton such as those bacterial groups belonging to the phyla *Proteobacteria* and *Bacteroidetes*. Large number of sequences mapping to marine invertebrate genomes is one of the intriguing results of our study where a clearer picture of periphyton diversity in terms of micro and macro inhabitants could be drawn. However, we also point out special concerns related to metagenome sequencing of environments involving communities of prokaryal and eukaryal species together. Functional repertoire of periphytic organisms comprises protein and enzymes related to biofilm's extracellular matrix, surface attachment strategies of primary invader species and various transporter systems, highly relevant for the biofilm mode of microbial life. Since metagenomics incorporates all organisms, regardless of size or metabolic capabilities, it can be a powerful tool in bioindicator research. However, the sequencing effort needed, and the associated bioinformatics analysis, is challenging. Still, we expect metagenomics to have an important role in the future of environmental monitoring.

Waste and Wastewater effluents: chemical and ecotoxicological characterisation (III)

131
Coupling in vitro and in vivo neurochemical-based assessments of wastewater effluents from the Maumee River Area of Concern (AOC)
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In this study we utilize *in vivo* and *in vitro* approaches to study whether wastes water treatment plant effluents released in the Maumee River (Toledo, OH) Area of Concern (AOC) contain neuroactive substances that may impair fish reproduction and behavior. Our approaches help extend the concept of endocrine disruption beyond routine bioassays (ER, AR, TH) under the premise that toxicants may also interact with and disrupt the function of neurotransmitter receptors and enzymes that play critical roles in vertebrate reproduction and behavior. Cell-free methods were used to study such interactions, and to also compare the *in vivo* and *in vitro* responses. First, 288 fish (fathead minnow) were exposed in cages to river water at 8 different sites along the Maumee River, including several in close proximity to wastewater treatment plant (WWTP) discharges. After 4 d of *in situ* exposure, brains were sampled and analyzed for N-methyl-D-aspartate (NMDA) receptor binding, and monoamine oxidase (MAO) and glutamine synthetase enzyme activity. The preliminary work shows that fish caged downstream of a major WWTP had increased MAO activity (66% and 35% in males and females respectively), and that NMDA receptor binding was also significantly changed (30% decrease in females). Second, *in vitro* studies were performed on river water extracts (final concentration 5x) to see if they interfere with the aforementioned neurochemicals studied *in vivo*. The initial *in vitro* results suggest that extracts optimized for recovery of alkylphenols significantly impacted the NMDA receptor binding (15% decrease), whereas those optimized for recovery of steroid hormones induced binding (up to 34%) in fish. Our work thus far suggests that wastewater effluents discharged to the Maumee River AOC contain chemicals that may directly interact (and possibly interfere) with neurochemicals that are important in fish reproduction and behavior. In addition, the work here (via a US EPA STAR grant) is taking next steps to identify key neurochemical indicators, resolve *in vitro* and *in vivo* responses, and compare responses across taxa.

132
Biomarkers of stress and effect evaluated on caged clams exposed to the Bay of Cádiz, SW Spain
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Effluents from waste water treatment plants (WWTP) constitute a continuous source of pollutants to the aquatic environment. Mussels are at high risk of exposure to such contamination because they can filter high volumes of water. The purpose

of this study was to examine sublethal responses and general stress on *Ruditapes philippinarum* after 14 days of exposure to field conditions in areas close to WWTP in the Bay of Cádiz, SW Spain. *In situ* experiments were performed with caged clams (duplicate) in Playa Chorrillos (reference site), La Puntilla and Trocadero located in the Bay of Cádiz. Subletal effects were evaluated *in vivo* in clam's haemolymph applying the lysosomal membrane stability test (LMS) by the neutral red retention time assay (NRRT). Enzymatic responses were studied in digestive gland tissues including biomarkers from Phase I: Ethoxyresorufin O-deethylaseenzyme activity (EROD) and dibenzylflourescein dealkylase enzyme (DBF); Phase II: glutathione S-transferase enzyme (GST); oxidative stress enzymes glutathione peroxidase (GPX) and glutathione reductase (GR), injurious effects were evaluated by lipid peroxidation (LPO) and DNA damage. After 14 day bioassay the mean NRRT (n = 10) in haemocytes from clams exposed to reference site P. Chorrillos was 128 ± 9 min, NRRT in clams exposed to El Trocadero was 50 ± 8 min indicating general stress syndrome, NRRT of clams exposed to La Puntilla was 33 ± 18 min, these clams were considered to present diminished health status. EROD and DBF enzymatic activities were significantly induced in EL Tocadero ($p < 0.05$); induction of DBF enzymatic activity might suggest the presence of pharmaceuticals in this area. GST enzymatic activity and antioxidant enzymes were significantly induced in clams exposed to La Puntilla and El Trocadero compared to reference site indicating oxidative stress. Injurious effects were observed in clams from EL Tocadero as LPO and DNA damage significantly differ from the response observed in clams from reference site ($p < 0.01$). Contaminants present in studied areas may affect the health status of *R. philippinarum* as well as other aquatic organisms. Finally, the data reported in this study including general stress and metabolic responses from Phase I and II represent important ecotoxicological information and provide useful reference for assessment of areas adjacent to WWTP and the effect their effluents might have in marine organisms, using as bioindicator species *R. philippinarum*

133
Characterisation of a sewage treatment plant effluent using biomarkers in brown trout (Salmo trutta fario) as tools in active monitoring
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The Neckar River (Southern Germany) represents an aquatic habitat which is strongly affected by numerous anthropogenic activities, thus it is an excellent system for ecotoxicological investigations. The present work is a model field survey for the assessment of the impact of sewage treatment plant (STP) effluents on fish health using active monitoring and biomarker techniques as suitable tools. During active monitoring, brown trout *(Salmo trutta fario)* from an uncontaminated fish farm were exposed in swimming steel cages up- and downstream of a Neckar River STP for 10 and 30 days respectively. Unexposed fish from the same hatchery served as negative control. The application of biomarker offered a possibility to investigate histopathological, geno-, proteo- and neurotoxic effects in various fish organs. Biological results were also complemented with chemical analysis data of tissues in order to provide a better understanding on exposure-effect relationships. Our results highlighted several histopathological abnormalities and an elevated stress protein level (proteotoxicity) in gills, liver, and kidney of brown trout at both monitoring sites. However, downstream fish always revealed stronger effects and also a higher mortality than the upstream ones. There were organ-specific reactions observed as well: gills showed a more rapid response to environmental contaminants when compared to liver and kidney, but neither toxic nor endocrine effects could be seen in the gonads. Our study did not reveal any genotoxic impact; the neurotoxic investigations are still ongoing. There were various organic compounds (PAHs, DDE) detected in brown trout tissue at both sampling sites in the low μg/kg range. Interestingly, an up to 4-fold concentration of disinfectant and synthetic musk derivates were found in the tissues of downstream-exposed fish, suggesting a considerable toxic input through the STP effluent. Our survey shed a light on an evident pollutant burden in the Neckar River; furthermore, it demonstrated the effectiveness of cage-exposure studies and biomarker methods in ecotoxicological effect characterisation.

134
Effect-Assessment of wastewater effluents by molecular biomarkers in brown trout (Salmo trutta)
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The identification and monitoring of chemical effects in organisms exposed in the environment is based on an array of approaches and specific endpoints. However, many of the existing tests have been established for only a few model organisms. Accounting for the variety of species and their different responses to environmental stressors is still a big challenge for ecotoxicological risk assessment. mRNA expression analysis of selected biomarker genes is a promising approach for field monitoring of non-model organisms because it can capture a wide spectrum of responses of organisms to chemical exposure.We established a biomarker gene set for brown trout (*Salmo trutta*) to assess the effects of micropollutants released by wastewater effluents. The biomarker set consisted of 20 genes which reflect different cellular stress responses like general stress (e.g. *Hsp70*), oxidative stress (*NrF2*) or biotransformation (*Cyp3a*). The transcriptional regulation of these genes was measured, using quantitative polymerase chain reaction (qPCR), in liver and kidney tissue samples of wild brown trout caught downstream and upstream of a wastewater treatment plant (WWTP) (St. Gallen, Switzerland) and from a reference river. qPCR results showed that mRNA levels of the selected genes were differentially regulated depending on the sampling site. Fish taken downstream generally had higher mRNA transcript abundances compared to fish from the upstream and reference sites, indicating that fish downstream are exposed to a higher level of environmental stressors. For example, the metal (*metallothionein B*) *or* endocrine disruption (*estrogen receptor alpha*) sensitive geneswere found to be significantly up-regulated in fish from downstream location compared to the other two sites. Results implicated a higher content of metals and potential estrogen-mimics downstream of the WWTP. Indeed, chemical analysis confirmed high concentrations of such compounds in the water from this site compared to the others. Partially, concentrations were above the threshold value for surface water. Our data demonstrate that the expression patterns of only ten fish per site already reflect the presence of certain contaminants. Therefore, transcript analysis of the biomarker set is a promising screening assay for assessing surface water quality. Moreover, based on the selected set of genes, predicting potential adverse effects may be possible, in particular for exposure to micropollutants.

135
Can Daphnia behavioral endpoints be used as tool for ecotoxicological assessment of wastewater effluents?
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Toxic stress can induce rapid behavioural changes in exposed organisms at concentration below acutely toxic levels. Analysis of behavioural parameters has therefore been proposed as tool for detection of potential toxicity in wastewater effluents. However, it is currently unclear how to derive robust ecotoxicological endpoints from observation of organism behaviour and how to quantify the sensitivity of these endpoints. The aim of the study is (i) to assess how behavioural responses can be used as ecotoxicological endpoints and (ii) to compare the sensitivity of standard versus behaviour endpoints in order to assess their usefulness for ecotoxicological assessment of wastewater effluents. As daphnid behavior shows a natural variability, different exposure concentrations and replicate testing is required in order to allow an appropriate statistical analysis of the behaviour parameters. However, commercially available image analysis systems have a limited test capacity (small number of measuring cells). A new image analysis system has been designed allowing the simultaneous tracking of up to 200 *Daphnia magna* distributed in 20 exposure chambers (10 daphnids per chamber). Twelve substances covering a wide range of different modes of action were tested at different concentrations with replicates. Two behavioural parameters (swimming speed and number of active organisms) were continuously recorded during 48 hours. Time courses of the two parameters were compared to control responses. Overall, significant behaviour responses are observed in the first hours of the experiment well below the acute EC50 (based on immobility at 48h) for most tested compounds. The behaviour endpoint "swimming speed" shows higher sensitivity than the number of active organisms. Differences between tested compounds are observed in the time of effect onset, duration and intensity of effects on swimming speed depending of their mode of action. Narcotics shows an intense increase peak of the swimming speed from the first hour of the experiment followed by a gradual decreased while neurotoxic chemicals induced a slightly but significant increase at different times depending on their mode of action. The exposition in the new image analysis system provides early and sensitive information compared to standard test endpoints and may be a great contribution to wastewater and effluent quality assessment

136
Evolution of stormwater quality between 2002 and 2010 for the city of Toulouse, France
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The city of Toulouse with its separate storm sewer system is ideal for studying stormwater. That is why since 2002, three stormwater sampling campaigns were conducted. Samples were taken from the outlets of two storm drains located in

heavily and moderately urbanized areas. Sampling was undertaken during wet weather and dry weather during the year 2002 for the first campaign, during the year 2007 for the second one and during the year 2010 for the last one. The overall pollution parameters were analyzed (chemical oxygen demand, biological oxygen demand, total nitrogen, ammonium, nitrate, total phosphorus, suspended solid matter, volatile suspended matter, pH, conductivity, turbidity). Results showed an evolution of stormwater quality between the three campaigns. They indicated also that dry weather had an impact on annual pollution load from separate storm sewer and that level of urbanization was also a factor.

Personal care products in the environment: strengthening science to support regulation

137

Occurrence, Distribution and Risk Assessment of Organic UV Filters in the Aquatic Environment in Hong Kong and Other Countries

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Organic ultraviolet (UV) filters protect skin against sunburn by absorbing UV radiation and are important ingredients in personal care products such as sunscreens, cosmetic make-up and lip care products. They can enter the aquatic environment through recreational activities and wastewater treatment plants (WWTPs). The increasing incorporation of organic UV filters into products has raised concern about their potential environmental impacts as some of these chemicals have the potential to bioaccumulate and have been shown to act as environmental estrogens. The occurrence and risks of these compounds in the aquatic environment (especially the marine environment) are not well known. The objectives of this study are to (1) develop quantitative analytical methods for the simultaneous multiclass determination of 12 commonly-used UV filters (4-MBC, BMDM, BP-1, BP-3, BP-4, BP-8, EHS, HMS, IAMC, OMC, OC and OD-PADA) in surface water, (2) determine their occurrence and distribution in samples collected in the United States (New York and Los Angeles), China (Hong Kong, Shantou and Chaozhou), Japan (Tokyo) and Thailand (Bangkok), and (3) conduct a preliminary environmental risk assessment of these compounds in the aquatic environment. Analytical methods for the extraction and quantification of the 12 UV filters were optimized based on solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). All of the target compounds were detected in Hong Kong seawater throughout the sampling period (July 2012 – June 2013) and were found to be widely distributed in the marine environment, with median concentrations less than 200 ng/L. BMDM, BP-3, HMS, OMC, OC, BP-1 and BP-8 were frequently detected (>75% of samples). The number of compounds detected was Hong Kong (12) > Tokyo (9) = Bangkok (9) > New York (8) = Los Angeles (8) > Shantou (5) = Chaozhou (5). The number of compounds detected generally increased with population density. Preliminary risk assessment showed that two frequently detected compounds (BP-3 and OMC) could pose high risk to egg development in fish and cause growth inhibition to algae.

138

Parabens in water and sediment: occurrence in river and lakes in different rural and urban environments

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Parabens are a group of alkyl esters of the p-hydroxybenzoic acid that are widely used as preservatives in pharmaceutical and personal care products. In order to gain knowledge about presence (or not) of parabens in different water categories, a screening study was performed in France and in 5 overseas territories in 2012. More than 1700 occurrence data were obtained in water and sediment samples for 3 parabens (methylparaben, ethylparaben and propylparaben). Samples were collected on more than 160 sites during 3 sampling campaigns (spring/summer/fall). Sampling sites representing different types of landcover were chosen. This screening study has been conducted on a larger datasets compared to previous reported literature data. For methylparaben and propylparaben, concentrations are similar in all samples, and no obvious effect could be identified associated to the type of anthropogenic sources. The occurrence of parabens as ubiquitous contaminats in almost 100% of the analysed samples can represent a risk. At the measured levels, acute and chronic toxicity is not expected with any of these compounds tested individually. However, currently available PNEC values do not take into account non-standard endpoints, such as endocrine disrupting effects which need to be considered in assessing the potential risksassociated to occurrence of parabens in the aquatic environment. Parabens are common hygiene product ingredients and special care must be considered for sampling.

139

Field dissipation and risk assessment of typical personal care products in biosolid-amended soils

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Antibacterial agents triclocarban (TCC) and triclosan (TCS), and synthetic musks AHTN (Tonalide) and HHCB (Galaxolide), and antifungal agents climbazole, clotrimazole and miconazole are extensively used in many household and personal care products. After use, they enter wastewter treatment plants and finally reach the receiving environment via discharge of effluent and disposal of sewage sludge. Due to their hydrophobic nature, these compounds have been detected in biosolids. Land application of biosolids has become a common practice throughout the world. However, concerns continue to be raised about the potential risks of this practice to the soil environment and public health. The aims of this study were to evaluate contamination levels of four typical personal care products (TCC, TCS, AHTN and HHCB as well as three antifungals) in biosolids and biosolid-amended soils of three field trial sites (Zhejiang, Hunan and Shandong), investigate their dissipation patterns in the biosolid-amended soils under different treatments (T1: single application; T2: repeated applications), and assess their potential risks to terrestrial organisms. The seven target compounds (TCC, TCS, AHTN and HHCB, as well as three antifungals) were detected in all biosolid-amended soils from the three trial sites (HN, SD and ZJ) in October 2010. The concentration levels for the seven compounds of both T1 and T2 in the biosolid-amended soils of the three sites were found to have the following order: TCC > TCS > AHTN > HHCB > miconazole > clotrimazole > climbazole. TCC was found to have concentrations ranging from 111 (ZJ) to 365 (SD) mg/kg for T1, and from 454 (ZJ) to1584 (HN) mg/kg for T2. The presence of these personal care products in the soils applied with biosolid indicates the persistence of these compounds in the soils. One-year field monitoring at the SD site showed dissipation of these chemicals with their half-lives of 51-1000 days. Preliminary risk assessment suggests that TCC and TCS might pose high risks to soil organisms based on the limited toxicity data, while AHTN and HHCB showed low to medium ecological risks. The results from this study will help evaluate the environmental impacts of these personal care products associated with biosolid application on agricultural land.

140

Determination of toxicity data of ultraviolet filters toward selected aquatic organisms for a preliminary environmental risk assessment

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UV Filters (UV-F) constitute a heterogeneous group of chemicals used as a protection against the harmful effects of the UV solar radiation. We can find them in a wide range of compounds such as personal care products or as additives in polymeric materials that need protection against sunlight. Nowadays, these emerging pollutants are considered persistent due to their continuous release into the environment [1]. Some studies performed mostly *in vitro* but also in *in vivo* have shown that many of these compounds can produce adverse effects on reproduction, interfering the normal development on fishes and rodents [2,3]. For instance, 4MBC has similar estrogenic effects than those of 17-b-estradiol in mammals and amphibious species [4]. Despite that, the knowledge on the potential chronic or acute toxicity to biota of these compounds is still very scarce [5,6]. In order to fill the knowledge gap on the ecotoxicity of UV-F, this study aims to i) determine the EC_{10},EC_{30},EC_{50} values of the target UV-F through a series of toxicological assays on three aquatic species i.e. *Daphnia Magna*, *Selenastrum Capricornutum* and *Vibrio Fischeri*, and ii) explore the potential synergetic effects in UV-F mixtures. Besides that, the ecotoxicological risks associated to UV-F and some of their known transformation products in selected case studies will be presented. **References** [1] Gago-Ferrero P., Mastroianni N., Díaz-Cruz M.S., Barceló D., J. Chromatogr. A 1294 (2013)106. [2] Weisbrod C.J., Zenker A.K., Kunz P.Y. Fent K. Toxicol. Appl. Pharmacol. 225 (2007) 255. [3] Klammer H., Schlecht C. Wuttke W., Schmutzler, C. Gotthardt I., Köhrle J., Jarry H. Toxicology 238 (2007) 192. [4] Kunz P.Y y Fent K. Aquatic Toxicology 79 (2006) 305. [5] Seeland A., Oetken M., Kiss A., Fries E., Oehlmann J., Environ. Sci. Pollut. Res. 19 (2012) 1781. [6] Kim J.W., Chang K.H., Isobe T., Tanabe S., J. Toxicol. Sci. 36 (2011) 247. Aknowledgments This work was funded by the Spanish Ministry of Economy and Competitiveness through the projects Nanotrojan (CTM2011-24051) and SCARCE (CSD2009-00065). The authors are members of the Consolidated Research Water and Soil Quality Group of the Generalitat of Catalonia, Spain, (2009-SGR-965).

141

Do insect repellents induce drift behaviour in aquatic non-target organisms?

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Synthetic insect repellents are substances applied to surfaces to discourage insects, mainly mosquitoes from landing on these surfaces. As some of these repellents have been detected in surface waters, they may also exert repellent effects on aquatic non-target organisms. In running water systems, aquatic invertebrates actively enter the drift in order to move out of locally malign environments. We thus tested the hypothesis that the widely used insect repellents DEET (N,N-Diethyl-m-toluaminide) and EBAAP (3-(n-n-butyl-n-acetyl-amino)-propionic acid ethyl ester) induce drift behaviour in the aquatic invertebrates *Gammarus pulex* (Crustacea, Amphipoda) and *Cloeon dipterum* (Insecta, Ephemeroptera), using a laboratory-scale drift assay. If the insect repellents cause drift behaviour in such non-target organisms at environmentally relevant concentrations, the resulting changes in resource consumption, but also in prey availability for predators, may influence ecosystem functioning in streams. Further, the effects of insect repellents on the behaviour of non-target organisms may also interfere with these organisms' natural behavioural responses to predator infochemicals with resulting detrimental effect of repellents in surface waters.

142

Screening human health risk of environmental and direct exposure to personal care products

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No existing assessment framework evaluates the uptake of product ingredients via both product use and via environmental exposure. To address this issue, this work presents a framework to flag potential risk and compare use and environmental exposures of chemicals within personal care products. The framework is based on the novel concept of the *product intake fraction* (*PiF*) defined as: the chemical mass of substance *i* within a product *x* taken in by humans via both use-phase and environmentally related emissions, per unit of chemical mass within that product. Chemical mass within products may be taken in by individuals via several use-phase pathways including direct dermal contact, and via several environmentally related pathways, such as food chain bioaccumulation. Applying the *PiF* conceptto evaluate all relevant exposure pathways for 25 chemicals within personal care products, we found use-phase *PiFs* ranged from 0.02 to 1 (kg intake/kg applied) and were mostly dominated by dermal intake, and environmental *PiFs* were generally several orders of magnitude lower and mostly dominated by intake of lipophilic chemicals with high bioaccumulation. *PiFs* may also be converted into daily population-scale intakes using production volume data. We demonstrated this framework using a paraben case-study with available NHANEs biomarker data for US women, and found model predictions generally in-line with observed *in vivo* paraben concentrations. To flag potential risk, the *PiF is* combined with Oral Equivalency Doses (OED) back-calculated from e.g. ToxCast *in vitro* bioactivity assays to estimate a maximum allowable fraction of chemical within a product. Evaluating the maximum allowable fraction for 25 chemicals within personal care products and to known product concentration data for 11 of the compounds (documented by the Household Product Database), we find 4 out of 11 potential chemical/product combinations with known chemical fractions within products exceeding the maximum allowable chemical fractions and 9 out of 10 with margins of exposure lower than 100. Evaluating both use-phase and environmentally related intakes of chemicals due to consumer products, suggests risk evaluation of dermal application of products must be held in high priority due to intakes that may exceed oral equivalency dose estimates for bioactivity and use-phase intakes generally dominating over environmentally associated intakes.

Fate and effects of nanoparticles under environmentally realistic conditions (III)

143

Sulphidation kinetics of silver nanoparticles reacted with metal sulphides

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Silver nanoparticles (Ag-NP) are used in many consumer products, most importantly textiles and cosmetics, due to the well-known antimicrobial properties of Ag^+. Eventually, these particles will be released from products and either reach surface waters or a wastewater treatment plant through the municipal sewer system. Ag-NP undergo either dissolution or other transformations, most importantly they react with sulphide to form Ag_2S. Because Ag as Ag_2S is several orders of magnitudes less toxic than ionic Ag^+, the sulphidation of Ag-NP is of prime interest. Thermodynamically Ag_2S is not the stable form of Ag in aerobic waters but it has been shown to be rather resistant towards oxidation with dissolved O_2, due to the formation of extraordinarily strong sulphide bonds. Therefore, the speciation of Ag-NP in aerobic surface waters is determined by the kinetic rate laws rather than by the thermodynamic stabilities. However, little is known about the sulphidation kinetics of silver nanoparticles under conditions typical for surface and waste waters. Although sulphide is dominantly present as bisulphide (HS^-, pH >7) in anaerobic environments, it is not stable under oxidizing conditions. Metal sulphides such as ZnS and CuS that resist rapid oxidation by O_2 may serve as sulphide sources for the sulphidation of Ag-NP under aerobic conditions. The goal of this study was, therefore, to assess the sulphidation kinetics of Ag-NP (10, 20, 40, 70 and 100 nm) reacting with ZnS and CuS. We measured the sulphidation kinetics of Ag-NP in oxygen saturated DI water buffered to a pH of 7.5. The reactions exhibited pseudo-first-order kinetics, with rate coefficients depending on the Ag-NP size and initial metal sulphide concentration. Analytical electron microscopy analysis revealed Ag-Ag_2S core-shell formation of Ag-NP. Reported concentrations of ZnS in aerobic surface waters range from 1.5 and 127 nM with corresponding half-life times of 5 days. By material-flow analysis predicted Ag-NP concentrations are 6.7 pM in combination with now experimentally determined half-life times of the Ag-NP ranging from 2 h to 2.4 days, therefore suggest a complete sulphidation of the Ag-NP also in aerobic surface waters.

144

Co-tolerance of microbial litter decomposers to silver nanoparticles and antibiotics

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With the accelerated production and use of silver nanoparticles (AgNP) in commercial products, freshwater ecosystems will most likely serve as recipients of, and repositories for, these nanomaterials. In addition to their potential environmental toxicity, there is a growing concern that AgNP act as a selective agent in antibiotic resistance. The aim of this study was therefore to evaluate the consequences of a long-term exposure to silver nanoparticles (AgNP), $AgNO_3$ (as a source of Ag^+) or a mixture of 5 antibiotics on the pollution-induced community tolerance (PICT) of microbial decomposers associated to leaf litter in freshwaters. Moreover, specificity of PICT was assessed by evaluating the co-tolerance between these toxicants. Bacterial production by ^{14}C-leucine incorporation and fungal sporulation rate by microscopy were used in short-term inhibition bioassays with antibiotics, AgNP and $AgNO_3$ to assess sensitivities of pre-exposed microbial decomposers. Diversity profiles of the bacterial and fungal communities following the different treatments were determined by DNA fingerprint technique. The results demonstrated that chronic exposure to AgNP, $AgNO_3$ or ATB induced structural shifts in the community and led to tolerance enhancements in the bacterial communities. On the other hand, communities exposed to AgNP also increased their tolerance to Ag^+ and ATB. Similarly, communities exposed to Ag^+ showed an increase of their tolerance to AgNP and ATB. Finally, bacterial community exposed to ATB displayed higher tolerances to AgNP and Ag^+. Overall, our findings support the fact that exposure to toxicants as diverse as ATB and metal-based nanoparticles may lead to similar effects when considering complex biological systems such as microbial litter decomposers raising a direct environmental and public health implications.

145

Fate and heteroaggregation of titanium dioxide nanoparticles in natural surface waters

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Despite continual progress in the development of engineered nanoparticles (ENPs), their fate and impact on the natural environment remain largely unknown due to challenges in detection and quantification. New strategies are necessary to more accurately assess ENP hazard and exposure. Titanium dioxide (TiO_2) NPs are of specific interest due to their use in a wide range of commercial products. The fate of TiO_2 NPs in aqueous environments depends on pH, ionic strength, and natural organic matter (NOM) concentration, however a lack of understanding regarding the behaviour of these NPs in natural systems remains, especially at relevant concentrations (μg/L range) where homoaggregation is less likely.[1] The heteroaggregation of TiO_2 NPs with natural suspended particulate matter (SPM) in the aqueous environment is also an important process in transport and fate.[2] Herein, we present the fate of TiO_2 NPs in natural waters from a lake with high NOM and low mineral SPM (Cholet, France), and a main river with high SPM and low NOM (Rhône River at Arles and Jons, France). The holistic approach presented here studies the fate of TiO_2 NPs under relevant concentrations in natural lake and river waters by characterization of their physical-chemical interactions and heteroaggregation with natural suspended matter (NSM). Homo- and hetero-aggregation kinetics were measured using light scattering, nanoparticle tracking analysis, and laser diffraction. The natural waters were evaluated for pH, ionic strength, NOM content, colloidal composition, and major and trace elements to elucidate the key contributors to NP fate. The kinetics of NP–NP and NP–NSM interactions were evaluated and the respective sticking efficiencies for homo- and hetero-aggregation were determined. They were influenced by the pH, ionic strength, and NSM composition of the waters. Nanoparticle concentration also

played a role in the heteroaggregation mechanism, assuming binding between neighbour mineral colloids. Heteroaggregation of the TiO_2 NPs varied significantly in the lake and river waters, thus suggesting different implications for NP fate. Increased mineral SPM in the river water favoured TiO_2 NP–NSM heteroaggregation and sedimentation. However, heteroaggregation was less evident in the NOM-rich lake water. Overall, this holistic approach details TiO_2 NP–NSM interactions pertinent to effective ENP risk assessment within natural surface waters.

146

Environmental fate models for engineered nanoparticles – simulating realistic conditions in a complex natural river system

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An increasing concern regarding engineered nanoparticles' (ENPs) environmental impact is derived from their increasing production, unconventional properties and mostly unknown behaviour in the environment. As of now, environmental concentrations of ENPs cannot be measured by in-situ quantification due to analytical challenges. Consequently, the design of meaningful toxicity tests and ENP risk assessment have to rely on rational estimations of ENP environmental concentrations. In this study we present the design and evaluation of an ENP fate model capable of incorporating environmental complexity to predict realistic environmental concentrations of ENPs. This model is based on a designated fate model for ENPs in surface waters first developed by Praetorius et al. in 2012. As in the original model, heteroaggregation between the ENPs and suspended particulate matter (SPM) is assumed to be a key process determining ENP transport. Realistic conditions were introduced by defining variable SPM composition and concentration along the river and time dependent emissions. We evaluated our model by investigating the predicted fate of nano-TiO_2 in the lower Rhône River (France) since this river is characterized by a unique SPM variability. In order to constrain our model with a realistic SPM composition, the SPM parameters employed were derived from a recent sampling campaign of that river. Since the attachment efficiency - α_{hetero}, a fundamental parameter governing the heteroaggregation process, is not easily measured, we evaluated our model's predictions for all possible α_{hetero} values between the measured SPM and nano-TiO_2. Our model results provide spatially resolved concentration values for the free nano-TiO_2 particles as well as the nano-TiO_2 bound to SPM, both in the water and sediment compartments along the course of the river. The combination of all results was then analysed to obtain the most probable transport profile of nano-TiO_2 in the lower Rhône This novel approach provides a powerful tool for predicting realistic ENP concentrations in surface waters, thereby enabling the design of rational toxicity tests and supporting the risk assessment process of ENPs.

147

Assessing the fate and effect of engineered nanomaterials in reference and wastewater derived organic matter

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While concern about the fate and effect of engineered nanomaterials (ENMs) in the aquatic environment is growing, few studies have assessed this under environmentally relevant conditions. Since a major pathway of ENMs to the environment is via wastewater treatment plants, this study assessed ENM behaviour and toxicity towards algae in the presence of secondary treated wastewater effluent. The studied ENM was widely used titanium dioxide (TiO_2), and its fate and toxicity in secondary treated effluent was compared to that in reference organic matter, specifically Suwannee River humic acid. Characterisation by laser diffraction indicated increased agglomeration of TiO_2 in the presence of the secondary treated effluent compared to the humic acid. Inductively coupled plasma-mass spectrometry confirmed the increased agglomeration leading to significant sedimentation by showing that titanium (Ti) concentration dropped to background levels after 24 hours in the wastewater, while only 25 % loss of Ti was observed over the duration of the algal toxicity test (72 hours) in humic acid. The algae toxicity tests showed that TiO_2 did not adversely affect growth, cell viability or photosynthesis ability at the studied concentration (1 mgL^{-1}). This was the case for both the humic acid and the secondary treated effluent, despite the difference in TiO_2 fate in the two types of organic matter. The studied concentration of TiO_2 was around 50 times higher than observed in secondary treated effluent, suggesting that at present TiO_2 from wastewater effluent does not pose a risk for algae. This study also highlights the importance of conducting ENM tests in environmentally relevant media, given the large difference in agglomeration and stability observed. Similar fate and toxicity experiments with zinc oxide are currently being undertaken.

148

Looking beyond standard testing of titanium dioxide nanomaterials – Consideration of relevant exposure scenarios

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Although several studies have investigated the effects of nanomaterials to different organisms, it is still unclear, if the test set up and endpoints of standard test guidelines are sufficient to describe the potential environmental implications of nanomaterials. Therefore, relevant exposure scenarios as e.g. the conduction of tests with solar radiation, mixture experiments of nano-TiO_2 and potential co-contaminants and the testing of embryonic development stages were considered during the conduction of OECD test guidelines with two nanoscale (NM 101, 7-10 nm and NM 102, 15-25 nm) and one non-nano scale TiO_2 materials (NM 100, 200-220 nm). *Daphnia* sp. acute immobilization tests (OECD 202, 10 fold diluted ISO medium) and activated sludge respiration inhibition tests (OECD 209) were performed under laboratory light (LL) and simulated solar radiation (SSR). Ti concentrations were measured in test vessels containing the EC50 concentration of the different TiO_2 materials (*Daphnia* tests). Acute and chronic mixture experiments with nano-TiO_2 and an organic biocide (triclocarban, TCC) were conducted with earthworms, activated sludge, daphnids and fish embryos (OECD 207, 222, 209, 202, 236). TCC concentrations were measured in soil samples of the chronic earthworm tests. In all tests single substances and untreated media (control) were additionally tested. Except for NM 101 (EC50 79.5 mg/L) in the *Daphnia* tests, the TiO_2 materials induced no toxic effects to any of the tested organisms when exposed to LL conditions. SSR enhanced the toxicity of NM 101, NM 102 and NM 100 (nominal/measured EC50 0.53/0.09, 1.28/0.24 mg/L, 3.88/0.33 mg/L). The measured EC50 of NM 102 is close to the predicted environmental aquatic nano-TiO_2 concentration in the μg/L range. Therefore, NM 102 may have environmental implications. Mixture experiments reveal that in most cases the presence of TiO_2 materials lowers the toxicity of TCC e.g. by 20 and 50% for fish embryos (NM 101, NM 102 at 1 mg/L) or 30 and 60% for earthworms (reproduction, NM 101 at 400 and 1000 mg/kg). The present study shows that SSR enhances the toxicity of TiO_2 materials, the presence of TiO_2 materials in most cases lowers the toxicity of the tested organic compound and the embryonic development of fish was not affected by TiO_2 materials. Summing up, to properly assess the potential environmental risk of TiO_2 materials it is necessary to consider relevant exposure scenarios, especially exposure to solar radiation.

Delving into the social and monetarised environmental impacts during the evaluation process of the Life Cycle of products in order to be able to take all three pillars of sustainability into account

149

Application of monetary valuation in Life Cycle Assessment: literature review and survey among practitioners

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Monetary valuation, or monetarisation, is the determination of the economic value of non-market goods, i.e. goods for which no market exists. Although monetary valuation has a great potential to be applied in Life Cycle Assessment (LCA), in particular in the weighting phase, several challenges limit its diffusion in the field, which resulted in only a few applications so far. This study evaluated different methods for monetary valuation with respect to their relevance in LCA. The specific objectives were: (i) to review systematically, analyse critically, and evaluate existing monetary valuation methods, as well as existing LCA-applications of these methods for converting (LCA) results quantified in physical units into monetary units; and (ii) to assess to what extent monetary valuation is used by the LCA community and what barriers limit its diffusion. After identifying and classifying monetary valuation approaches, methods, and LCA applications via a keyword-based literature review, their key features, strengths, and weaknesses were determined. Monetary valuation methods and their LCA applications were then evaluated according to a comprehensive set of criteria including: *Scientific foundation; Documentation; Completeness; Uncertainty; Complexity; LCA relevance & compatibility.* Two web-facilitated surveys were performed to collect primary information on: (i) the extent of monetary valuation use in LCA; (ii) which monetary valuation methods are used; and (iii) the opinions of the practitioners and decision makers. LCA practitioners and consultants were identified by using publicly available lists of practitioners, via scientific associations, the network of the project participants, and popular LCA forums. The critical review encompassed 8 monetary valuation methods and 12 applications for LCA. For the two surveys, the total number of respondents was 209. The critial review showed that observed- and revealed-preference methods and the abatement cost method have limited applicability in LCA, whereas the conjoint analysis method and the budget constraint method are the best options for monetary valuation in LCA. The web-based survey among LCA practitioners showed a large interest in and support for monetarisation in LCA, but also a smaller group of respondents with legitimate objections to monetarisation. The surveys revealed a need for education and information about what monetarisation is and does.

150

External costs of air pollution from energy supply: Reviewing methodologies from ExternE to NEEDS

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European air quality policies are regularly supported by scientific impact assessments, including the monetary valuation of environmental and health damages, also referred to as environmental damage costs (EDC). Since the early 1990s, related methodological developments have taken place in the Externalities of Energy (ExternE) project series and follow-up activities. This study aims at looking back on more than 15 years of external cost quantification in Europe and discusses the main methodological evolutions from the 1990s until recently. The focus here is on the variability of human health costs caused by classical air pollutants (mainly NO_x, SO_2, particulate matter). Using a case from the energy sector, major influencing parameters are identified: exposure modelling is shown to lead to variations in results of up to 30% between different assessment frameworks. Concerning risk functions and monetary valuation, changes in assessing mortality risks due to long term exposure together with assumptions on particle toxicity explain most of the observed variability. This variability and an increasing use in policy making require continued scientifc efforts for a further improvement of the underlying methodology. As regards the integration of EDC into Life Cycle Sustainability Assessment, the Impact Pathway Approach as implemented in the EcoSenseWeb model provides indicators at the endpoint/damage level and this in a monetary and non-monetary way (i.e. physical). However, the physical indicators would benefit from an aggregation, e.g. in terms of DALYs, for integration into environmental LCAs, while the monetised damages could be directly integrated in societal Life Cycle Costing.

151

The external cost of air pollution in the canton of Zurich and the cities Zurich and Winterthur

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In order to justify and allocate public spending on the reduction of air pollution, policymakers are interested in the external cost of air pollution in total as well as per source of pollution. Therefore, we were given the mandate to estimate these external costs in the canton of Zurich and the cities of Zurich and Winterthur. Using existing knowledge of concentration-response relationships, we firstly assessed the effects of primary fine dust, nitrogen oxide, ozone, and ammonia on human health, maintenance of buildings, biodiversity, forests and crop. Secondly, we estimated the monetary value of these damages, hence external cost of air pollution in total. In the last step, the external costs of air pollution were attributed to their respective sources, which are traffic, agriculture, industry as well as households and services. We find that the external cost of air pollution in the canton of Zurich was 882 Mio. CHF in 2010. Between 2000 and 2010 air pollution could be reduced. If this had not been the case, the external cost of air pollution would have been 146 Mio. CHF higher in 2010 than it actually was.

152

Assessing effects of life cycle on income distribution and infant mortality at national level

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We propose a method to anticipate the effect of the monetary flows generated by a life cycle's production step on income distribution and infant mortality at the national level. The method builds on a cause-consequence relationship identified as an impact pathway. Implementing it requires a two-stage process. The first to which most attention is given here, consists in measuring the change in the income distribution within the country due to the change in monetary flows induced by the production step. The second consists in using an elasticity coefficient estimated econometrically to predict the potential variation in infant mortality due to the variation of income distribution (in Bocoum et al., 2013, SETAC Glasgow). A way of assessing the monetary flows created by the changes in the life cycle is to use Input-Output (IO) analysis, which is based on interdependencies between economic branches and sectors. In this case study, we consider the project of substituting 40 million hectolitres of locally produced table wine to the same imported volume in country A, a theoretical OECD country. We use a national IO table (fictional but that represents a certain economic structure) to infer the variations at the production step and translate them into numbers of extra employees in different sectors, using productivity ratios. After passing out the new employees in the different classes of salaries, we compare the new income distribution in the different classes to the previous one, and calculate the Gini index. This index usually serves as a measure of income concentration. In the case study, we find that the envisioned change decreases income inequality from 0.39 to 0.37, in country A. The effect on infant mortality is inferred from the variation of income distribution and from the above mentioned elasticity coefficient. The 0.02 point decrease of income inequality decreases infant mortality from 11 per 1000 to 10.3 per 1000, fourteen years later. We set many hypotheses. We therefore suggest comparing only effects that are obtained using the same procedure. The pathway is only suitable for life cycles generating large-scale income flows. Development of other impact pathways highlighting other socioeconomic mechanisms is required. Paying more attention to activities downstream of production in future researches is also essential. **Key words:** Life cycle – income distribution – population health – impact pathway

153

Anticipating the psychosocial factors effects in social LCA

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The public and private decision-makers are showing an increasing interest in the environmental and social consequences. However, in the social field, a method equivalent environmental LCA remains largely to be built. The purpose of the work is to set up a new theoretical model, and an assessment grid, to allow us to anticipate damage to health of workers (involved in the life cycle) caused by psychosocial risk factors at work. We will take into account improvements caused by the positive psychosocial factors too. However, we do not study the effects of pollutants, radiations and other dangerous items, because they have been dealt with in environmental LCA (for some models) in order to account for the "Human health" impact. We study frequent risk factors that are linked with the organisation of work itself. There is a "human cost of stress in the workplace", which is different between the various sectors and the different ways of organizing work. The work is based on an unconventional approach. In fact, traditionally, health is considered ex post and based on personal risk factors of individuals. The anticipatory approach of the work, on the other hand, identifies groups of workers considered to be at risk because of psychosocial risk factors to which they are exposed. The outputs of the work will contribute to anticipate damages to health. They will help decision makers to choose the best scenario for the production organisation as far as possible. The expected results from the work also will strengthen the research program in the social life cycle assessment in order to provide a new "method of evaluation" (or "pathway") between psychosocial factors of the work and health of workers engaged in the life cycle of the company.

154

Using simplified S-LCA to identify socially responsible purchasing criteria

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Introduction\nSustainable purchasing is a growing global trend. Increasingly organizations from both private and public sectors are integrating social and environmental criteria into the purchasing process as a means to reduce their environmental footprint, leverage social benefits and foster a sustainable economy, throughout a product's life cycle. But as "a management process used to acquire goods and services in a way that gives preference to suppliers [and products] that generate positive social and environmental outcomes" (Sustainability Purchasing Network 2007), sustainable purchasing must, to be effective, rely on sound sourcing criteria – for each dimension of sustainability.\nOn the environmental side, organizations have today access to a vast array of resources, such as fact sheets, to help them selecting the products and/or suppliers with the highest performance in a life cycle perspective. Based on LCA results, hotspots are identified and specific purchasing criteria, such as certifications, can be proposed to manage risks or reduce potential impacts. To date, tools and resources are, however, much more limited to cover the socioeconomic dimension of sustainable purchasing. Consequently, most organizations rely either on detailed supply chains analysis, adopt generic tools such as suppliers' codes of conduct or require indifferently social certifications such as SA8000 from their suppliers. While useful, these tools are time and resources consuming or non-specific and hence, less effective.\nTo overcome this limitation, ECPAR (Espace québécois de concertation sur les pratiques d'approvisionnement responsable) has commissioned Groupe AGECO to develop a method to integrate in a structured and rigorous way the socioeconomic dimension to technical sheets for sustainable sourcing. Based on a simplified Social LCA (S-LCA) methodology, this method has been developed and implemented in the specific case of paper products. The objective of this communication is to describe this methodology, present the results and discuss the opportunity of using simplified S-LCA as a tool to identify sound sourcing criteria in a sustainable purchasing strategy. Bibliography\nSustainability Purchasing Network. 2007. Sustainability Purchasing Trends and Drivers. 64 pages. Key words\nSustainable sourcing; Simplified S-LCA; Social purchasing criteria.

Current Developments and Challenges on Sediment toxicology in Scientific and Regulatory Contexts

155

Advances in setting principles for regulatory sediment risk assessment

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The European Chemicals Agency (ECHA) hosted a Topical Scientific Workshop on Risk Assessment of the Sediment Compartment in May 2013. Key areas of sediment risk assessment in a broader regulatory context were discussed. The Workshop aimed at updating scientific principles and guidelines for sediment risk assessment by facilitating dialogue between academia, regulators, the regulated industry and other stakeholders. Experts agreed that there has been a significant development regarding the scientific principles and basic methodologies applicable to prospective and retrospective assessments. These developments and new paradigms should be considered for updating guidelines for assessing ecological risks of chemical substances for freshwater and marine sediments. In order to understand the state of the art of the sediment risk assessment in the field of industrial chemicals, ECHA has carried out a critical analysis of the sediment information submitted under REACH. For the substances registered under the 2010 registrations deadline long-term sediment toxicity testing is a standard information requirement according to Annex X section 9.5.1. Of the 2010 dossiers less than 10 % include a sediment toxicity study whereas an adaptation of the standard information requirement is given in the majority, circa 90 %, of the dossiers. For dossiers submitted in 2013 sediment toxicity is not a normal standard information requirement and only 4 % have submitted experimental sediment toxicity information. Overall the data shows that the sediment information submitted under REACH is a compilation of available information generated for two main purposes: 1) pollution control, mostly for the marine environment, and 2) generic (e.g. (pre)-marketing) assessments), based on standardised, mostly fresh-water, species, with limited number of species and functional traits. Based on the Workshop outcome and the analysis of the sediment information in REACH registration dossiers, it was possible to identify the most critical areas where information is needed for the scientifically-justified and consistent assessment of risks in the sediment compartment. The clear need for a more complete coverage of the complex sediment environment for a better quality sediment risk assessment will be discussed.

156

Nickel toxicity in freshwater sediments: Developing an integrated effects assessment to comply with challenges posed by REACH and the Water Framework Directive

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The protection of the sediment ecosystem is embedded in regulatory frameworks such as the European Union regulatory initiative for the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and the EU Water framework Directive (WFD). In general the limited availability of appropriate sediment toxicity data and the lack of understanding of how certain sediment characteristics may modify contaminant bioavailability hamper the process of determining risk to the sediment compartment. This study presents a comprehensive effort to develop predictive, mechanistically based sediment effects guidelines for nickel that can be applied across broad ranges of sediment chemistry. A robust database of chronic toxicity tests was generated using a total of 11 field collected sediments spiked with 5 concentrations of nickel and tested with a suite of 9 benthic organisms representing a variety of feeding strategies and taxonomic groups. The data were used to populate a species sensitivity distribution (SSD) and to derive a realistic worst case Predicted No Effect Concentration (RWC-PNEC) for nickel. In a second phase 6 invertebrate taxa were used to characterize relationships between nickel toxicity and sediment characteristics. Bioavailability models were derived based in EC20 values for selected low-variation endpoints. Most of the obtained toxicity thresholds for nickel in sediment were predominantly correlated with acid volatile sulfides (AVS). The strength of the correlations (indicated by the slope) was less pronounced for those organisms with a subsurface feeding/burrower behaviour (slope: 0.12-0.20) if compared to those species which can be considered surface deposit feeders/swimmers (slope: 0.36-0.49). The bioavailability models were subsequently used to establish a SSD for a RWC sediment low in AVS concentration (0.8 μmol/g dry wt.). The results of support the basis of the SEM-AVS concept for nickel as no toxicity was observed at SEM-AV S < 0 for all species. Furthermore, at increasing AVS concentrations nickel toxicity was mitigated for all species, but the extent of this effect was dependent on the living /feeding strategy of the organism. An HC5-50 of 136 mg Ni/kg dry wt. was estimated from the log-normal function calculated with ETX. This value was deemed appropriate for a RWC PNEC or sediment quality guideline (SQG) because it reflects conditions that represent the 10th percentile of parameters controlling nickel bioavailability.

157

AVS based bioavailability models are inadequate for predicting risk in oxic sediments

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The partitioning of metals to solid-phases can reduce the bioavailable fraction of metal. Under anoxic conditions, reduced sulfur and organic carbon are the primary binding fractions for metals; however, these fractions are not as important under oxic conditions. Our research aims to improve metal bioavailability models for stream ecosystems by assessing the role of oxic sediments in sequestering metals. Sediments were spiked with Cu or Ni and equilibrated under anoxic conditions. These sediments were aged in a flow-through mesocosm (100+ days) while concurrently exposing Hyalella azteca to measure changes in toxicity. Temporal sampling produced a fine-scale understanding of geochemical and toxicological dynamics in the sediment as surface sediments oxidized. As sediments aged, oxygen penetration depth increased. Through time, total metal pools remained stable but porewater metals declined as metal speciation changed. Further, sediment toxicity decreased through time until it did not differ from controls. Declines in toxicity occurred concurrently with increased pools of metal (particularly for Cu) associated with crystalline Fe and Mn oxides. These data suggest that the current bioavailability models may be inadequate for sediments with oxidized surface sediments and future research should focus on developing bioavailability models that incorporate Fe and Mn oxides.

158

Bioaccumulation assessment by battery testing allows read across among marine benthic invertebrate species

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In ecological risk assessment schemes, empirical tests are crucial but not sufficient because regulatory context also requires mechanistic understanding of chemical exposure pathways and effects. Relatively few studies address read across of chemical exposure between benthic invertebrates. The main objective of the present work was to improve understanding of uptake mechanisms for a range of species with different traits, and to model these processes. A secondary objective was to test a novel whole-sediment test set up, which combines test species in one aquarium separated by gauze. Using this set up, bioaccumulation tests with PCBs and chlorpyrifos were performed with the following marine benthic invertebrate species: *Arenicola marina*, *Corophium volutator*, *Macoma balthica*,and *Nereis virens*. Exposure conditions were varied using spiked standard OECD sediment with low, medium, or high organic matter content. Biota sediment accumulation factors for PCBs were *N. virens* < *A. marina* ≤ *M. balthica*< *C. volutator*. The relative importances of uptake by either pore water or sediment ingestion were estimated by modelling. Multispecies test designs performed better than individual test designs due to better water quality.

159

Considering cohesive sediments for the chemical and ecologi-cal status of aquatic ecosystems – New lines of evidence for a WoE approach based on nematodes

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Toxicants accumulated in cohesive sediments impair the functioning of the benthic community that holds the fundamental ecosystem service of closing the nutrient cycle by metabolizing the deposited substrate. Moreover, contaminated sediments represent a permanent source of pollutants for the water phase. Thus, polluted cohesive sediments can impede the achievement of a good chemical and ecological status of the ecosystem and should therefore be considered for the quality assessment of waterbodies. However, most of the monitoring tools and pollution indicators cannot be applied to cohesive sediments, as they are based on macro-invertebrates that do not represent the dominant group in these substrates. Therefore, indices were developed to assess the chemical and ecological status of cohesive sediments that are based on the *in-situ* nematode fauna inhabiting these sediments: (1) Effect-based Sediment Quality Guidelines (SQG) providing thresholds for sediment concentrations of contaminants protecting the nematode fauna in cohesive sediments (SQG_{Nema}, Brinke et al., 2013, SETAC EU Meeting, Glasgow, UK, Abstract No. 502), (2) the NemaSPEAR[%]-index evaluating the ecological status by assessing the abundance of Nematode SPEcies At Risk (Höss et al., 2011, Environ Int 37: 940-949). Both indicator tools can be used as lines of evidence (LoE) in a nematode-based Weight-of-Evidence (WoE) approach for sediment quality assessment. In the presented study, these new indices were validated by defining a NemaSPEAR[%] threshold value to draw a line between a good and moderate ecological status and by testing this threshold with contaminated field samples and comparing the NemaSPEAR[%] with SQG_{Nema} and SQGs based on macro-invertebrate data (SQG_{Makro}). Moreover, microcosm experiments were conducted to reveal responses of the NemaSPEAR[%] to single chemicals. The results showed that a NemaSPEAR[%]-threshold of 30% was able to identify sites with polluted sediments, while the NemaSPEAR[%] correlated well with mean TEC-Q values using SQG_{Nema}. The microcosm study showed a dose-dependent response of the NemaSPEAR[%] to Zn confirming the indicative power of this index for chemical pollution. Finally, a WoE approach combining these two LoEs was applied to a large data set of fine river sediments. This allowed prioritization of sites concerning management actions and confirmed the value of these nematode-based indices for quality assessments of potentially polluted waterbodies.

160

Assessing the environmental quality of estuarine and coastal sediments from the Atlantic and Mediterranean regions using different in-vitro bioassays

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Sediment contamination poses a potential risk for both ecosystems and human health. Approaches to biomonitor sediment quality are essential in order to characterize the health status of aquatic environments and, ultimately minimize threats and prevent the adverse effects to aquatic wildlife. However, risk assessment remains a challenge as sediments contain complex mixtures of toxicants, and traditional chemical analyses can neither provide information about potential hazards to organisms nor identify and measure all present contaminants. This work deals with the combined use of (a) the fish hepatoma cell line (PLHC-1) and (b) gonad subcellular fractions from sea bass (*Dicentrarchus labrax*) for the screening of cyto-, repro- and genotoxicity as well as the presence of CYP1A and oxidative stress inducting agents in sediment organic extracts from estuarine and coastal areas (Atlantic and Mediterranean regions). Extracts of those sediments collected near harbours, industrial effluents and untreated sewage showed significant cytotoxicity (Alamar Blue and CFDA-AM assays) and genotoxicity (micronuclei induction) in PLHC-1 cells. This was often associated with a higher presence of CYP1A inducing agents (induction of EROD activity after 6- and 24 h-exposure) and generation of oxyradicals in PLHC-1 cells. Furthermore, the incubation of sediment extracts with sea bass gonad subcellular fractions (mitochondria and microsomes isolated from testes and ovaries) allowed the detection of those extracts that significantly inhibited the synthesis of androgens (CYP17, CYP11b) and/or estrogens (CYP19) in sea bass, indicating the presence of endocrine disrupters. Overall this approach based on the combination of different in-vitro bioassays provides useful information to discriminate between polluted and less impacted areas, and highlights those sediments that could pose risk to benthic organisms and that require further action to improve their environmental quality. Moreover, the differential response of the applied assays to the different extracts emphasized the importance of employing multiple endpoints for the determination of specific modes of action of complex mixtures of contaminants.

Novel approaches to incorporate in vitro bioassays in risk assessment

161

Conceptual Framework to Derive Effect-based Trigger Values for Cell-based In-vitro Bioassays

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Bioanalytical tools have been applied for monitoring of water quality as well as assessing treatment efficacy of wastewater and advanced water treatment for many years. Cell-based assays either assess general cytotoxicity or target a specific mode of toxic action or step on the toxicity pathway. Comprehensive risk assessment thus requires a battery of bioassays to cover a range of modes of toxic action and/or toxicity pathways relevant to the water sample to be tested. Lack of trigger values and interpretation guidelines have hampered greater adoption in (regulatory) monitoring applications. In this paper we present a conceptual framework for the derivation of effect-based trigger (EBT) values. We propose to translate existing chemical guideline values to EBT values. It must be noted that there is no single EBT value but an individual EBT value must be defined for each bioassay and each set of guideline values. Depending on the biological endpoint, we differentiate between two approaches to define EBT values: (1) For receptor-mediated effects, where a reference chemical can be defined with clear maximum and minimum potency, the corresponding bioanalytical equivalent concentrations (BEQ) can be used to define effect-based trigger BEQ (EBT-BEQ). (2) For non-specific toxicity and adaptive stress responses, where the BEQ concept does not apply, mixture toxicity concepts can be applied to derive effect concentrations of the sample in units of relative enrichment factors that equate to the triggers, i.e., effect-based trigger effect concentrations (EBT-EC). Per definition the EBT-EC must include considerations of the chemicals' interaction in mixtures. The proposed approach was tested with the Australian Drinking Water Guidelines and the Guidelines for Water Recycling and applied to a set of samples collected at Australian wastewater, water reclamation and drinking water treatment plants. EBT-BEQs were derived for a number of endocrine endpoints and in case of estrogenicity also for several bioassays covering the same mode of action. EBT-ECs were derived for non-specific cytotoxicity and for oxidative stress response. All reclaimed and drinking water samples were compliant with the proposed trigger values. The proposed framework is an initial step towards the development of effect-based trigger values. As a next step, the research outcomes must be discussed with competent authorities in order to implement them in practise.

162

Applicability of in vitro assays for assessment of estrogenicity in case studies – advantages and uncertainties

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In vitro bioassays assessing potential of compounds to activate estrogenic receptor are one of the most commonly used and studied type of bioassays. Main aim of this presentation is to show the positive examples of utilization of estrogenic bioassays in monitoring of waste and surface waters as well as to discuss uncertainties regarding their utilization as risk assessment tools such as possible contribution of various types of compounds to estrogenicity of surface waters. An interlaboratory study comparing concentrations of polar organic compounds in passive samplers placed in one sewage treatment effluent showed high variability of concentrations of steroid estrogens determined by chemical analysis in different laboratories and demonstrated usefulness of utilized estrogenic in vitro assay. Estrogenicity and steroid estrogens were also monitored in samples from 75 different European WWTPs. Steroid estrogens were not detected by instrumental analysis above LOQ 10 ng/L in any sample whereas in vitro assay was more sensitive and showed estrogenicity above 0.5 ng/L in 27 samples. Results of the in vitro assay also allowed comparison of estrogenicity of WWTPs of different capacities and wastewater sources. The third study demostrated estrogenicity in all 7 tested headwaters flowing through areas of the Czech Republic with minimal anthropogenic pollution. Increased estrogenicity was detected in all samples from locations downstream of the first towns with WWTPs compared to samples collected at upstream locations. In case of municipal WWTPs, steroid estrogens seem to be mostly responsible for detected estrogenicity and therefore the authors think that trigger values can be derived from in vivo PNECs and in vitro relative potencies of these compounds in the bioassays. However, information about compounds responsible for estrogenicity as well as for other specific modes of actions in rivers is limited compared to what is available for WWTP effluents or rivers close to their discharges. The potential contribution of some less studied compounds occuring in surface waters to estrogenic activity detected in vitro as well as the uncertainties and limitations in interpretation of in vitro data in will be disscussed. Acknowledgements: Supported by the Czech Science Foundation grant P503/12/0553

163

Contribution of dyes on the mutagenicity found in Brazilian surface water: the Piracicaba River case

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Dyes are used in the coloration of different substrates, but the largest use is on textiles industries. Dyes are emergent contaminants and have being found in the environment, especially in Brazil, India and China. Recently mutagenic dyes and aromatic amines were detected in samples of Carioba Wastewater Treatment Plant (WWTP) effluent and Piracicaba river at Americana, SP, Brazil. This region has the largest textile center of Brazil and Carioba WWTP receives effluents from various textile industries. But some industries discharge their effluents directly into the Quilombo river, which is a tributary of Piracicaba river. The aim of this study was to analyze the occurrence of dyes in surface water under the influence of textile industries discharges and in treated effluent from a Waste Water Treatment Plant that receives textiles effluents; and evaluate the mutagenicity of these samples and

the contribution of dyes in the mutagenicity found. Samples were collected in Piracicaba river before WWTP discharge, Quilombo river, Carioba WWTP treated effluent, and Piracicaba river after WWTP. Samples were organically extracted by liquid-liquid using dichloromethane/methanol (2.5:1 v/v) and analyzed by a Linear Ion Trap Quadrupole LC-MS/MS. Extracts were tested in the Salmonella/microsome assay using microsuspension method with strains TA98 and YG1041, in the absence and presence of exogenous metabolic activation (S9). Ten different disperse dyes were detected in surface water and effluent samples of Americana city, SP State, Brazil. The most frequent dyes were Disperse Red 1, Disperse Red 60, Disperse Blue 60, Disperse Blue 291, Disperse Blue 373, and Disperse Violet 93. Samples were mutagenic in Salmonella/microsome assay and the highest potencies were found after the discharge (Quilombo river and treated effluent) with YG1041 in the presence of metabolic activation. The dyes Disperse Blue 373, Disperse Blue 291, Disperse Red 1 and Disperse Violet 93 are contributing to the mutagenicity found in Quilombo and Piracicaba River. But the dyes Disperse Blue 373 and Disperse Violet 93 are the major contributors corresponding almost to 40% in Quilombo river and 30% in Piracicaba river after the discharge, both cases with YG1041 with S9 in the second sampling. Other dyes and aromatic amines were found in the samples and studies to evaluate their contribution on mutagenicity are being conducted. Acknowledgement: Fapesp (2008/10449-7 and 2012/13344-7)

164

Bioluminescence as a tool for studying mechanisms of toxic effects

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Bioluminescence (BL) is glowing of living organisms; it is based on chemiluminescent enzymatic reactions. Marine luminous bacteria are widely used as bioassays for monitoring environmental toxicity. Main testing physiological parameter here is BL intensity. The BL assay systems are based on biological objects of different levels of organization – bacteria-based or enzyme-based bioassays, providing for a study of the effects of toxic compounds on cells or enzymes, respectively. Basing on a broad investigation of effects of model toxic exogenous compounds on BL assay systems, classification of the effects on the BL enzyme reaction was suggested. The effects were classified as physical, chemical and/or biochemical ones. Five mechanisms are discussed: (1) change in electron-excited states' population and energy transfer, (2) change in the efficiency of the S-T conversion in the presence of an external heavy atom, (3) change of rates of coupled reactions, (4) interactions with enzymes and variation of the enzymatic activity, (5) nonspecific effects of electron acceptors. The broad experience in investigation of effects of exogenous compounds makes the BL assay systems to be a very convenient tool for studying toxicity mechanisms. The BL assays were found to be sensitive to alpha- and beta-radionuclides. The role of peroxides and electron transfer in hormesis and toxic effects of radionuclides was studied. Changes in protein secondary structure in bacterial cells exposed to radionuclides was discussed in terms of a stress response of the bacterial cells to the low-dose chronic radioactivity. The mutagenic effect of tritium was studied using restriction analysis of marker amplicons. Detoxification of solutions of metallic salts and organic oxidizers by humic substances (HS), products of natural transformation of organic matter in soils and bottom sediments, was studied. Detoxification mechanisms were revealed to be complex, with chemical, biochemical, and cellular aspects conditioning those. The detoxifying effects were explained by: (i) decrease of free toxic compound' content in water solutions under binding and redox neutralization by HS, (ii) increase of rates of biochemical processes in the bioassay system under HS influence, (iii) enhancement of mucous layers on cell surface as a cellular response to unfavorable impact of toxicants. Mechanisms (ii) and (iii) revealed an active role of the bioassay systems in the detoxification processes.

165

Flagging health risks of chemicals by combining in vitro bioactivity data with environmental and consumer product exposure modeling

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Combining *in vitro* bioactivity data with exposure models is essential to predict potential public health risks. We present a tier 1 framework to flag chemical exposures of potential risk based on *in vitro* bioactivity data for 229 chemicals from the US EPA. To understand chemical-specific exposure, we refined thousands of chemical categories in the US EPA ACToR/CpCat product database to harmonize with exposure modeling. Most chemicals matched several of the following exposure categories: direct intakes (e.g. food), dermal application (e.g. cosmetics), pesticides (e.g. ingestion of residues), and indoor and environmental emissions. Results were also cross-checked with the Household Product Database and FDA-approved food additives. We independently estimated population-scale chemical intake fractions due to environmental and indoor emissions and product intake fractions using three multi-media models (USEtox, CalTox, RAIDAR). Model results predicted similar trends within two orders of magnitude for most chemicals. Intake fractions were multiplied by emission estimates or production volumes to extrapolate daily intake of chemical per unit bodyweight (mg/kg/day). Modeled intake doses per category varied greatly across chemicals due to physicochemical properties and emission estimates - the main source of uncertainty. We found using conservative approaches, that chemical intake due to consumer product use generally exceeds intakes due to environmental exposures, with the greatest intake being 2,600mg/kg/day for phenol in a personal care product. Population-scale average intake doses due to environmental exposures were always less than 0.34 (mg/kg/day). When comparing intake estimates with the bioactivity data, we found 66 chemicals with a maximum intake that exceeds the minimum oral dose equivalents for observed biological activity. For these 66 chemicals there were several chemical/exposure classification combination that exceeded min oral dose equivalents: 12 cases due to environmental exposure, 20 cases due to product use within home, 41 cases due to personal care product, and 47 cases due to food ingestion. These chemical exposures are flagged as needing to be further evaluated in order to understand possible public health threats.

Pollinator risk assessment: past, present and future

166

Second tier options in the EFSA Guidance Document on Risk Assessment of Plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)

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Pesticides are considered as one factor among others that are contributing to the decline of pollinators. Moreover, the current risk assessment schemes for pesticides are not considered to be able to address the risk to pollinators in a comprehensive way. This indicated the need to review the current risk assessment schemes and to develop new, more sophisticated ones. As a response to this regulatory challenge, the European Commission tasked EFSA to develop guidance for pesticide risk assessment for bees. The new guidance document was issued on July, 2013, but has not yet been adopted for use in regulatory risk assessments. The guidance document suggests the implementation of a tiered risk assessment scheme with a simple and cost-effective first tier moving to more complex higher tiers (e.g. using field studies). Each tier of the risk assessment ensures that the appropriate level of protection is achieved. However, the guidance document was heavily criticised by different stakeholders including the industry and some European regulatory bodies. One of the main concerns raised by the industry was the severity of the first tier together with the unfeasibility of the highest tier options. However, there has been very little discussion regarding the 2nd tier options, which is between the 1st tier and highest tier level. A number of 2nd tier options are suggested by the guidance document which can be used to refine the estimation of the exposure of pollinators to pesticides when foraging on the treated plants. These options can also be used to provide a more realistic oral exposure estimate for larvae. Crop or compound specific data are needed at this tier, with which a low risk may be achieved. Consequently, there will be no need to perform the more complex and expensive field studies. As an example, it is expected that using compound specific information on residue levels in pollen and nectar will provide a solution in many cases. For other cases data on the sugar content of the nectar will be sufficient. The presentation will deliver an overview of the most important 2nd tier options that are included in the guidance document. Also, a case study will be presented explaining how some of these options can be applied in practice.

167

Development of a Toxicokinetic-Model of the Bee Hive.

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Pollination is an important factor of the food economy and the honey bee *Apis mellifera* is the most important commercial pollinator. Over the last few years, potential effects of insecticides on bee colonies have been discussed. There is largely consensus that the risk assessment for bees will benefit from a deeper understanding of mechanisms of bee exposure to pesticides. To understand the effects of toxicants in the bee hive it is important to fully understand their fate within bee colonies. Literature data were analysed to determine the relevant toxicokinetic processes in the bee colony and how these processes influence the fate of chemical substances in the bee hive. To realistically estimate the quantity of chemical substances bees are exposed to within the hive, the whole process of resource collection and processing has to be taken into account. We assume bee-to-bee-contact as the most important factor for the distribution of chemical substances in the hive; physicochemical properties of the materials in the hive have to be considered, as well. A conceptual model, which is inspired by PBTK-models, was developed; it comprises all relevant processes. It is the basis for a model simulating bee hive toxicokinetics. This model is implemented in R. To solve the differential equations (DE) the model uses the package deSolve, that is developed for solving ordinary DEs. The model will provide a deeper understanding about toxicokinetics and exposure in the bee hive.

168

Weeds in the treated field – a realistic scenario for pollinator risk assessment?

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In July 2013 the European Food Safety Authority (EFSA) released its final guidance on the risk assessment of plant protection products (PPPs) to bees (EFSA, 2013). One objective of the guidance was to produce a simple and cost effective first tier risk assessment scheme to ensure that the appropriate level of protection is achieved (EFSA, 2013). However, recent impact analyses have indicated that the first tier of this risk assessment does not act effectively as a screen for compounds of low risk to bees. An example of a non-toxic herbicide (honey bee acute oral and contact LD_{50} >85 & >200 μg a.s./bee respectively) is presented here and screened through the first tiers of the EFSA risk assessment. In this example a worst-case exposure to flowering weeds inside the treated field is highlighted. If realistic farming practices (e.g. tillage) are considered weeds are not usually prevalent in fields before and shortly after sowing, in addition to this the application of the example herbicide will likely remove any weeds which remain shortly after application. It is therefore suggested that the scenarios in the guidance could be considered overly conservative and in some instances unrealistic. The EFSA guidance suggests that if < 10% of the area of use is flowering weeds then the exposure route is not relevant in the 90th%ile case. However, despite this, the option to generate data or refine based on available data is questioned as no guidance for the assessment of the abundance of weeds is available (EFSA, 2013). We present and discuss the use of empirical evidence (e.g. occurrence and growth stage of weeds in control and herbicide treated plots from efficacy field trials conducted for regulatory submission) to illustrate that the scenarios in the guidance document could be modified using currently available data to create a more effective screening step and still ensure that the appropriate level of protection is achieved.

169

Glyphosate: evaluation of exposure and effects on honey bee brood (Apis mellifera) development

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To address new European Union (EU) data requirements for plant protection products, honey bee risk assessments are required where exposure of adults and larvae via direct contact or from residues in nectar and pollencannot be excluded. Acute oral/contact toxicity studies are performed on adult bees and registrants may also be required to conduct Tier 1 larval chronic toxicity studies for which an OECD guidance is still under development or Tier 2 colony-level brood effects studies. For EU re-registration of glyphosate, potential exposure and effects, on honey bee brood/colonies were assessed in separate studies. To quantify exposure, a greenhouse study involved a spray application of a glyphosate formulation to flowering *Phacelia tanacetifolia* during peak bee foraging. Glyphosate concentrations over time in forager-collected pollen and nectar were analysed. Mean glyphosate levels in nectar were >10X lower than in pollen and declined rapidly with DT_{50} values of 1-2 days. Pollen and nectar residue values were used as inputs to a bioenergetics-based exposure model to establish realistic worst case dose levels. To quantify effects on brood/colonies, a Tier 2 bee brood feeding study was performed using the Oomen test design. Colonies were tested at four dose levels including the control. Colonies were assessed 1 week prior and weeks 1, 2 and 3 after dosing. Assessments tracked development of individual larvae and emergence, and the health of the colony as a whole with exposure confirmed by residue analysis of larvae collected from within the colony. No effects at any dose level consequently the No Observed Effect Level for brood development and adult survival was the highest dose tested, providing a sufficient margin of safety on the risk of glyphosate to honey bees. This conclusion is consistent with results of independently performed semi-field and field bee brood studies using a glyphosate-based formulation.

170

A four year field program investigating long term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam

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Neonicotinoid residues in nectar and pollen from crop plants have been implicated as one of the potential factors causing the declines of honey bee populations. Indeed the European Commission has introduced a 2 year moratorium for several uses of imidacloprid, thiamethoxam and clothianidin on bee attractive crops. However, much of the data that has implicated neonicotinoids in the decline of honey bee health have been generated either under laboratory conditions or using unrealistic exposure conditions. Field studies are a credible methodology to assess any effects under real world conditions. In this field study the long-term risk to honey bee colonies was investigated following four years consecutive single treatment crop exposures to flowering maize and oilseed rape grown from thiamethoxam treated seeds at rates recommended for insect control. During the study honey bee mortality, foraging behavior, colony strength, colony weight, brood development, food storage levels and over wintering success were monitored and reported. The results confirmed a low risk to honey bees from systemic residues in nectar and pollen following the use of thiamethoxam as a seed treatment on oilseed rape and maize. These results contribute towards reducing the gap in our understanding of exposure and risk to honey bees from the use of neonicotinoids as seed treatments under field conditions.

171

Uptake and translocation of imidacloprid to nectar and pollen in sunflower and raspberry

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Imidacloprid, a neonicotinoid insecticide known to be highly toxic to bees, is allowed for use in greenhouse production. Recently, imidacloprid was found in nearly all samples from waste (up to 7 μg/g dry weight) and in leachating from waste (up to 48 ug/L) from greenhouse flower production (Roseth 2012, Roseth pers. communication 2013). The Norwegian Food Safety Authority initiated a project to investigate if bee-attractive plants grown in waste with imidacloprid-residues, e.g. waste from greenhouses production, can take up and translocate imidacloprid to nectar and pollen. Sunflower and raspberry were selected as relevant bee-attractive plants for this study. The study was performed in greenhouse in six growth tubes (each 6 m) with drainage pipe, leca and peat. Confidor (active substance imidacloprid) solved in water was prayed to the growth media in two layer; one in the midle and one top surface. Imidacloprid was found in all nectar and pollen samples from both plant species grown in peat with and initial concentration around 28 μg/g dry weight. The project was financed by the Norwegian Ministry of Agriculture and Food's 'National Action plan for reducing risk from the use of pesticides'.

Fish model species in environmental toxicology (I)

172

Comparison of experimental versus predicted acute toxicity data of four polar narcotic analogues in the zebrafish embryo

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In line with the 3R principles, several modelling methods use physico-chemical properties of chemicals to categorize them into different mode of action (MOA) categories and to predict their toxic potency. Despite the benefits of these modelling methods, several studies have demonstrated inconsistencies between predicted and experimental toxicity. Transcriptomics offers opportunities to elucidate the underlying biological mechanisms of toxicity which can result in more detailed chemical classification and subsequent toxicity prediction. As zebrafish embryos are not considered test animals until 120 hours post fertilization (hpf) according to European legislation, they can be used in alternative testing strategies and offer great potential in biological MOA classification. In this study, QSAR modelling, and morphological and microarray analysis of zebrafish embryos were used to evaluate the predicted toxicity and the mode of action of four aniline analogues which only differ in their degree of chloro-substitution: aniline; 4-chloroaniline (4CA); 3,5-dichloroaniline (3,5DCA) and 2,3,4-trichloroaniline (2,3,4TCA). Results demonstrate a positive relation between the toxicity and the log K_{ow}(increasing chlorination) which is in accordance to the ECOSAR QSAR prediction.The toxicity of aniline with a low log K_{ow} value, however, was overestimated resulting in a wrong categorization. Since chlorinated anilines are structural analogues with a designated polar narcotic mode of action, similar toxicity responses are assumed by modelling tools. Both morphological data (recorded at 24 and 48 hpf) as well as transcriptomics data (custom made Agilent 15k microarray at 48 hpf) demonstrated that the tested chlorinated aniline analogues have a similar mode of action. Overall, oedema, the absence of blood circulation and abnormal pigmentation were detected for all tested chemicals. The mode of action involved alterations in heme biosynthesis, electron transport chain, structural integrity and cell transport, with more specific toxic mechanisms being observed when chlorosubstitution increases. This study demonstrates that the

toxicity of chemicals can be predicted for zebrafish embryos by QSAR models showing greater toxicity with increasing chlorosubstitution. However, biological data at the morphological and molecular level provide more insight in the general mode of action as well as more specific effects associated with the different structural analogues.

173

Effect of an herbicide, ethofumesate, on aerobic metabolism in roach (Rutilus rutilus) at two temperatures

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Ethofumesate is a benzofuran herbicide commonly used to control weeds of sugar beet. Its mechanism of action in plants is to inhibit the synthesis of very long chain fatty acids. The aim of this study was to determine the effect of ethofumesate on aerobic metabolism in juvenile roach at different (biochemical, molecular and cellular) regulation levels. Studying the energy metabolism constitutes an appropriate approach to detect physiological disturbances of organisms linked to their exposure to pollutants. Indeed animal survival depends on the availability of energy necessary to ensure physiological functions as maintenance, growth and reproduction. Among biological processes involved in cellular energy synthesis, we focused on glycolysis and respiratory chain pathways. Two temperatures were tested to assess potential effects of this parameter on energy metabolism responses to chemicals. During exposure, fish were exposed to 0.5; 5 or 50 $\mu g.L^{-1}$ of ethofumesate during seven days, at 10°C for the first experiments and at 17°C for the second. For each experiments, fish were sacrified and white muscle sampled at 0,1 and 7 days. Glycolysis fluxes, gene expression, electron transport system, anti-oxydant responses and mitochondria ultrastructure were analyzed. Concerning glycolysis pathway, aerobic flux seemed to be affected at T_1 when individuals exposed to ethofumesate at 10°C presented a lower aerobic flux than control. Expression of HK gene decreased significantly at T_1 in fish exposed to ethofumesate at 10°C. As HK is the only aerobic enzyme of the glycolysis pathway, we can hypothesize that such an under-expression of this gene could be related to an effect on aerobic flux. Focusing on respiratory chain, this pathway was differently affected depending on temperature. Ethofumesate seems to have more effects at 17 than 10°C, what seems consistent, as energy metabolism is known to be more active with increasing temperature. This temperature effect was observable in electron transport system and antioxidant activity essentially. Indeed, ETS decreased in muscle of contaminated fish at 17°C only. In conclusion, this study revealed a disturbance on aerobic metabolism due to ethofumesate exposure on juvenile roaches, especially in glycolysis pathway with an effect of exposure temperature. This study provides potential new biomarkers related to the energy metabolism, but further analyses are required to understand the action mechanisms.

174

A novel method for measuring disruption of energy metabolism in vivo in the zebrafish

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In recent years halogenated phenolic compounds (HPCs) such as hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been found in many marine species from the Baltic Sea, e.g. cyanobacteria, red algae, blue mussels and a variety of fish species. Although these substances seem to be primarily natural products they can also be produced by in vivo metabolism of PBDEs. It has been shown that many marines species (e.g. algae, cyanobacteria and marine sponges) are able to produce OH-PBDEs. The amount of product formed depends on environmental factors like temperature, light, wind or nutrient supply. Variations, e.g. due to eutrophication and climate changes might increase the levels of OH-PBDEs produced Recent studies have shown that one of the most ubiquitous OH-PBDE found in marine fish, 6-OH-BDE47, is very toxic to zebrafish Danio rerio embryos. This developmental toxicity is, at least in part, due to potent inhibition of mitochondrial oxidative phosphorylation (OXPHOS), thereby disrupting the main source of cellular energy. In this study, we hypothesize that other HPCs may also modify energy metabolism and that this effect might explain some of the severe health effects observed in Baltic Sea wildlife, such as wasting syndrome, decrease in fat content and reproductive insufficiency (e.g. low hatchability in herring roe). To this end we have developed a novel method for monitoring the disruption of OXPHOS in vivo in a small fish species (Danio rerio). The potential to disrupt OXPHOS of 19 commonly found HPCs was studied in vitro and in vivo. Our results indicate that many HPCs found in marine species have OXPHOS disrupting potency. Mixture experiments indicate strong synergistic effects.

175

Genomic and gene expression responses to genotoxic stress in zebrafish (Danio rerio)

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Sublethal exposure to genotoxicants in the environment may influence genome integrity in affected organisms and potentially cause permanent alterations in the genome, thus posing the need to more adequately estimate not only DNA damage but also its longevity. Therefore, in this study we assessed primary DNA damage and existence of potentially persistent DNA alterations in the zebrafish genome using three different models (PAC2 cell line, embryos, adults) upon exposure to model genotoxic agents and the following recovery period. Genotoxicity assessed using Comet assay served as an initial screening for evaluation of general impact on DNA integrity under genotoxic stress. To test for genomic effects upon genotoxic stress quantitative RAPD and AFLP were applied. Furthermore, expression of a suite of genes involved in DNA repair, oxidative stress response and xenobiotic metabolism was monitored in order to get better overview of zebrafish genome responses to genotoxic stressors. Additionally, AFLP method was applied on adult specimens one year after their exposure to genotoxicants at larval stage in order to further evaluate longevity of observed DNA alterations. Comet assay detected DNA damage in all three zebrafish models, which in some cases persisted upon recovery period as well. DNA alterations assessed using qRAPD and AFLP were observed even in cases when Comet assay indicated lack of significant damage proving their sensitivity for detection of various DNA alterations that remain undisclosed by the Comet assay, such as DNA adducts, mutations, structural DNA changes and changes in DNA methylation pattern. Zebrafish larvae proved to be the most sensitive zebrafish model for revealing genotoxic effects, which was evidenced by the high incidence of DNA alterations and the lack of significant DNA repair at the early stages of larval development. Some of the genomic alterations observed in larvae were still detectable in the adulthood, indicating the formation of persistent genomic modifications. These results thus underline zebrafish larvae as particularly sensitive model in such surveys and encourage the simultaneous use of various models and methods for more accurate evaluation of the consequences of the genotoxic insult.

176

'Mode-of-action' of human pharmaceuticals in fish: The effects of ibuprofen on Fathead minnow (Pimephales promelas)

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The widespread detection of human pharmaceuticals in the aquatic environments has raised concerns over the potential eco-toxicological effects in non-target organisms. Ibuprofen is a widely used prescription and over-the-counter medicine with an estimated reported usage of ~260,000 Kg in England. To evaluate whether or not ibuprofen poses a risk to fish (fathead minnow, FHM) we tested the 'read-across hypothesis' that stipulates any potential effects will be related to the MoA of ibuprofen, and that these effects will only be seen when plasma concentrations in fish are similar to human therapeutic plasma concentrations (Cmax). Ibuprofen alleviates pain, fever and inflammation by inhibiting the biosynthesis of prostaglandins (PGs) through inhibition of the enzyme (the target) Cyclo-oxygenase (Cox). There are two mammalian isoforms of Cox (1 and 2). The Cmax of ibuprofen is between 15-25 mg/L following a 400 mg dose. Cox gene fragments were identified and characterised in FHMs and we demonstrated that FHMs possess an additional Cox isoform to mammals (Cox 1, 2a and 2b), similar to the closely related zebrafish (Danio rerio). We established that FHMs exposed, via the water for 72-96 hours, to ibuprofen concentrations of 270 and 370 μg/L resulted in plasma concentrations within the Cmax. Uptake of ibuprofen was rapid, however large individual variation was observed, suggesting that drug metabolism in fish, as in humans, is variable. The effects of ibuprofen exposure on FHM tissues were examined by measuring Cox gene expression using 'quantitative real-time polymerase chain reaction' (qPCR) in control and exposed fish. Since PGs are the products of Cox activity, Prostaglandin E metabolite (PGEM) concentrations were also measured using an ELISA assay (Cayman Chemicals, USA). Contrary to expectation, Cox gene expression was increased in exposed fish compared to the control, which may indicate compensatory up-regulation if the enzymes have been inhibited following ibuprofen exposure. However, PGEM concentrations were significantly decreased in exposed fish, compared to the controls (*Unpaired t test, P< 0.0001*). Our data do not suggest that ibuprofen would present an immediate risk to fish at environmental concentrations, however as PGs have been linked to roles in fish reproduction, more work is required to determine the significance of PG inhibition in wild fish.

177

Physiological effects of exposure to ibuprofen in Menidia beryllina.

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Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) that is often detected in wastewater effluent and in the receiving waters. Ibuprofen has a relatively short half-life and therefore is not persistent in the environment; however its continual introduction into the aquatic environment via municipal wastewater effluent can lead to some fishes experiencing chronic exposures in areas affected by wastewater. Ibuprofen is a non-selective cyclooxygenase inhibitor, which can result in reduced prostaglandin production. Prostaglandins are involved in regulating reproductive hormone production and complex spawning behaviors, among other physiological processes, therefore exposure to ibuprofen may adversely impact fishes. We used inland silversides (*Menidia beryllina*; 66 days old), an estuarine fish species that is a potentially useful indicator of contaminant exposure in estuaries throughout North America, to determine the physiological effects of acute and chronic exposure to ibuprofen on gene expression and reproductive output. Acute 96-hr exposures were used to identify the effects of ibuprofen on survival, which was 98-100% after 96 hr for concentrations as high as 18.8 mg/L and subsequently decreased at higher concentrations. Juvenile fish were then exposed to one of three ibuprofen exposure treatments (0.025, 0.25 and 2.5 mg/L), along with an experimental control group, for 14 days. We used qPCR analysis to determine the effects of ibuprofen on the expression of genes involved in prostaglandin synthesis, arachidonic acid metabolism, steroid synthesis and hormone receptors. We found that ibuprofen affected the transcript levels of several genes involved in prostaglandin synthesis and arachidonic acid metabolism. Additionally, we found that the expression of hormone receptors was non-monotonic and appeared to be more affected by ibuprofen at the lowest exposure concentration than at the higher concentrations. We will use a species-specific microarray to further examine the transcriptomic responses to ibuprofen exposure. Our results show that at low concentrations, chronic exposure to ibuprofen can induce cellular responses in *M. beryllina*. We detected these cellular responses at concentrations that resulted in reduced reproductive output in adult *M. beryllina*, which suggests a potential link to a whole organism-level impact of exposure to ibuprofen.

Wildlife ecotoxicology: from acute toxicity to low level, chronic exposure related effects (I)

178

An innovative large scale field study to investigate unintentional effects of plan protection product on the grey partridge Perdix perdix reproduction in cereal ecosystems

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Unintentional effects of current plan protection products (PPPs) use on non target animals have been little documented *in situ* on vertebrates living in cultivated farmlands. In this context, we investigated the effects of exposition to PPPs on the grey partridge *Perdix perdix* reproduction. This declining gamebird, typical of cereal ecosystems, is exposed to PPPs during its whole life-cycle and thus appeared as a relevant indicator. We used an innovative approach to characterize the potential exposition to PPPs, combining daily radiotracking of > 400 females in spring & summer and a survey of farmers to know the farming operations they carried out on each of their field plots (*i.e.* PPP used, date of application, dose used, etc.). Birds were monitored twice a day to record their status (surviving, dead or missing) and their locations in crops. Locations were reported on a map of crop cover using a nomad GIS. Clutches (ca. 280) were located once the incubation had started. The biological material (pipped eggshells of hatched eggs, destroyed and intact eggs) was collected after clutches had hatched or failed. Hence we could record *in situ* several reproductive endpoints: clutch fate, cause of failure, clutch size, egg hatching rate. We determined the status of intact eggs (infertile *vs.* dead embryo) and the development stage of dead embryos as well as any potential body defect. In addition, we measured eggshell thickness. We identified the crop plots frequented by females during the incubation, laying and pre-laying periods using the convex envelop of daily locations on QGIS. Then, thanks to the farmer's questionnaire, we identified the PPPs spread on those crop plots for each period. This procedure is relevant from an ecological point of view since it both tooks the spatio-temporal variability in PPP use and partridge habitat use into account. We replicated this field work over 12 study sites to consider a diversity of situations. They were located in north-central France, a region of intensively cultivated farmlands where the grey partridge is still present as wild populations. After a presentation of the whole methodology used, we will characterize the potential direct/indirect exposition of eggs to the different PPP types and present statistics correlating reproductive endpoints and potential exposition to > 100 chemicals. This research is funded by the *Pesticides-Ecophyto 2018* program of the French Ministry of Environment.

179

Chlorophacinone toxicity and threshold for coagulopathy in American kestrels (Falco sparverius)

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Recent studies with captive American kestrels (*Falco sparverius*) and Eastern screech-owls (*Megascops asio*) found these raptorial species to be considerably more sensitive to the first generation anticoagulant rodenticide (FGAR) diphacinone than traditional avian test species (mallard *Anas platyrhynchos*, bobwhite *Colinus virginianus*). Using data from these studies, both deterministic and probabilistic risk assessments indicate that diphacinone is far more hazardous to raptorial birds than previously recognized. Chlorophacinone (CPN) is a FGAR that is widely used for the control of pest species in urban and suburban settings, agriculture and island restoration projects. We examined its toxicity in adult male American kestrels fed diets of rat tissue mechanically-amended with CPN and rat tissue containing biologically-incorporated CPN (derived from lab rats fed CPN bait) for 7 days. Nominal CPN levels in these diets were 0.15, 0.75 and 1.5 μg/g food wet weight, and actual concentrations were analytically verified as being close to these target values. Neither food consumption nor body weight were affected by CPN, and exposure and adverse effects were dose-dependent. There were no dramatic differences in toxicity between the mechanically-amended and biologically-incorporated CPN diets. Overt signs of intoxication and anemia were apparent in kestrels receiving 1.5 μg CPN/g food. Both prothrombin time (PT) and Russell's viper venom time (RVVT) were prolonged at doses of 0.75 and 1.5 µg CPN/g compared to controls and the 0.15 μg CPN/g group. Histological examination of tissues revealed evidence of hemorrhage in many CPN-treated kestrels (but not controls), and CPN residues in liver were dose-dependent, averaging about 0.2 μg/g wet weight at the greatest exposure levels. Using benchmark dose software, dietary thresholds at which PT and RVVT were prolonged in 10% of the kestrels corresponded to exposure rates of approximately 70 and 26 μg CPN/kg body weight per day, respectively. Liver CPN concentrations at which PT and RVVT were prolonged in 10% of exposed kestrels were about 0.12 and 0.07 μg CPN/g wet weight, respectively. These data are being used to more fully assess the hazard of CPN to non-target raptorial birds.

180

Exposure of bearded vultures to external antiparasitics used to treat livestock

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Veterinary drugs that are commonly used in livestock production can remain as residues within the carcasses of animals after death. As a result, various scavengers, including vultures, may be exposed to a range of toxic compounds – the implications of which remain very poorly characterised. Across Asia, exposure to diclofenac through this pathway has caused the near global extinction of at least three species of Old World *Gyps* vultures. This highlights the urgent need for far better pharmacovigilance and the requirement for improved, more comprehensive life-cycle assessments for the myriad of pharmaceutical products currently in use. Here we present data regarding the exposure of bearded vulture (*Gypaetus barbatus*) to external antiparasitics in Spain. We have studied 24 suspected poisonings of bearded vultures and the breeding failure of one pair in Aragon (NE Spain) between 2006 and 2013. As evidences were found that external antiparasitics used on livestock were a probable cause of some of these poisonings, 24 feet of pork and 116 feet of lamb were analysed by GC-MS. Brain acetylcholinesterase (AchE) activity was measured in the brain when this sample was available. One of the bearded vultures showed diazinon (12 ng/g) in the gastric content, another one showed permethrin (56 ng/g), and lamb feet associated with two other poisonings showed diazinon (28-29 ng/g). Brain acetylcholinesterase activity in the bearded vulture with diazinon in the gastric content was 16.8 μmol/min/g, which is lower than the activity found in another bearded vulture dead by traumatism with 28.5 μmol/min/g. Pork feet had no residues of external antiparasitics. On the contrary, lamb feet showed residues of diazinon, pirimiphos-methyl, chlorpyrifos, fenthion, permethrin or cypermethrin, with occurrences varying among slaughterhouses (up to 100%). In some cases, lamb feet contained up to 618 ng/g of diazinon and 1,008 ng/g of cypermethrin. Washing the feet with water significantly reduced the levels of external antiparasitics, so this measure was regularly adopted to reduce the risk of exposure in the managed feeding sites. We can estimate an exposure in bearded vultures of up to 0.05 mg/kg, which is lower that the LD_{50} calculated for wild birds of 2 mg/kg. However, this exposure is almost chronic and some evidences exist that organophosphates at levels of a few μg/g can affect serum AchE, metabolism and the immune function of birds.

181

Does pollution increase pathogen infection in terrestrial wildlife?

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Among the stressors organisms are exposed to, pollutants have been recognised as having deleterious effects on the immune system. Subsequent variations of immunocompetence may facilitate the infection of organisms by pathogens. We studied the relationships between trace metals (TMs) and various pathogens in the house sparrow *Passer domesticus*, the wood mouse *Apodemus sylvaticus* and the lesser horseshoe bat *Rhinolophus hipposideros*. The composition of the landscape was quantified because it may have a great importance on both pathogen transmission and pollutant transfer in the environment. Pathogens in house sparrows were studied in 5 populations along an urbanization gradient in France. Small mammals were sampled from 7 variously polluted sites around a former smelter in northern France. Droppings of bats were collected in 25 maternity roosts in Franche-Comté region (northeastern France). Pb concentrations in sparrow feathers were positively associated with urbanization while Cd and Zn concentrations were positively associated with forest cover. In mice, prevalence of the bacteria *Anaplasma phagocytophilum* and of the coccidia *Giardia spp*. was related to landscape composition. Richness of coccidia increased with natural forest and decreased with urban areas. The global pathogen richness increased with increased presence of hedgerows and copses and also with landscape diversity. In bats, Cd, Cu, and Zn concentrations and individual pathogen richness were positively related to the percentage of forest cover. *P. relictum* prevalence in sparrows was negatively associated with Cd concentrations but positively associated to Pb. In mice, the prevalence of *Giardia spp*. and *Eimeria spp*. significantly increased with Pb concentrations in kidneys. *Giardia spp*. prevalence and the pathogen richness were positively associated with Cd and Pb concentrations in soils. In bats, prevalence per roost for *E. hessei* was positively related to Cd concentrations. Individual pathogen richness was related to both essential and non-essential TMs. For 3 different animal models and several pathogens having different transmission modes, relationships were found between one and/or several pathogens and one and/or several TM concentrations in tissues. Relationships between pathogens and TMs are complex and modulated by landscape features. Underlying mechanisms and impacts on wildlife are still to be elucidated and constitute promising perspectives within the field of stress ecology.

182

Levels and Risk assessment of DDTs in biota samples from the Ethiopian Rift Valley Region

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Despite restrictions and bans on the use of organochlorine pesticides, developing countries are still using them for agricultural and public health purposes. In African countries suffering from malaria epidemic, an exemption was made possible as DDT is relatively cost effective, easy to produce and highly effective for control of malaria through indoor residual spraying (IRS). Among those countries, Ethiopia is one of the major importer and consumer for the past four decades with a problem of obsolete pesticides. Approximately, 400 metric tons of DDT is used for IRS per year to control malaria throughout the country. Therefore, this study is designed to investigate the levels and risk assessment of DDTs in biota samples from the Ethiopian Rift Valley Lakes - Lake Awassa and Lake Ziway. Surface sediment samples (n=25), and 49 fish samples of Tilapia (n= 20), Catfish (n= 18) and Barbus (n= 11) from Lake Awassa; 105 fish specimens of Tilapia (n = 27), Zillii (n = 19), Carp (n = 27), Catfish (n = 27) and Barbus (n = 5), from Lake Ziway and 23 bird individuals belonging to Hamerkop (n = 5), Sacred ibis (n = 7), Marabou (n = 6), and Pelican (n = 5) resides around lake Ziway were collected. Muscle tissues were taken for analysis. ΣDDTs in surface sediments from Lake Awassa were found in the range of 3.64 to 40.2 ng/g dry weight and high levels of DDTs were observed in the vicinity of the inflow side and from samples next to a village close to agriculture areas. Reidue levels of DDTs in fish samples from Lake Awassa were ranged from 0.63–73.2 ng/g ww in the order of: Barbus > Catfish > Tilapia. The levels of ∑DDTs from Lake Ziway ranged from 0.77–61.9 ng/g ww and 114 to 1600 ng/g ww in fish and bird species, respectively. Generally, *p,p'*-DDE was the predominant congener. Evaluation of risk assessment showed a concern of toxicity for human health and bird population. Assessment based on the cancer risk estimates set at a carcinogenic risk level of 10^{-6} were in the area of concern span and the calculated hazard ratio of DDTs exceeded the threshold value of one, indicating daily exposure to DDTs is a potential concern. The main DDT metabolite, *p,p'*-DDE had significantly high burden in bird species, which may be sufficient to cause adverse effects on eggshell thinning and survival of young birds.

183

Increased temperatures and pesticide exposure: a double threat to population of amphibians? A microcosms experiment on embryos development

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Amphibian populations are declining worldwide, and the increase application of pesticides in agricultural activities may be one factor contributing for the decreasing abundance of this group. Climate changes are expected to constitute an additional threat to amphibian populations being also able of enhancing the toxicity of some chemicals. Thereby, the present work aims to use microcosm experiments to study the combined effects of temperature and pesticide exposure on the normal embryo development of amphibians. To assess the developmental effects of these factors, embryos of *Pelophylax perezi* (Iberian green frog) were submitted simultaneously to three temperature regimes (18, 23 and 25 ºC) and three concentrations of the pesticide FORUM C - active ingredients: dimethomorph and copper oxychloride - (0.5, 0.75 and 1.125 mg L^{-1}). Embryos in midblastula (stage 8) were used to start the test and throughout the assay growth, survival and malformations of the tadpoles were evaluated. Basic water quality parameters (D.O., pH and temperature) were measured in the beginning, before renewing the test solution and at the end of the test. Each temperature test finished once every tadpole in the control reached the 25th stage of development. Besides mortality, growth and teratogenesis parameters, a battery of antioxidant biomarkers including Glutathione peroxidase (GPx), Glutathione-s-transferases (GSTs) and Glutathione reductase (GRed) were assessed. In addition lipid peroxidation (LPO) was also determined at the end of the assay in order to assess oxidative damage. These biochemical parameters provide further comprehension of the subcellular effects resulting of the tested combined exposure, allowing to understand some toxicity pathways. The results of these experiments using the combination of two stressors will provide a more realistic overview of the threats faced by amphibian populations in the wild. This information is of crucial importance to anticipate the effects of chemical pollution in the rapidly changing environment.

Keywords: Microcosms experiments, amphibians, developmental toxicity, biomarkers

Innovations in environmental analytical chemistry: the quest for pollutants at trace levels (I)

184

A novel approach to study human habits through mass spectrometric analysis of urban wastewater

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The chemical analysis of urban wastewater for the excreted biomarkers of endogenous human metabolism is a potent approach for monitoring habits and lifestyle of an entire community. Mass spectrometry is the strong base of this novel approach since this technique is sensitive and specific enough to detect analytes at trace levels even in a complex matrix such as raw urban wastewater. This study presents several applications of this approach performed in Italy both at local and national scale. The aim was to estimate temporal and spatial trend of illicit drug use, monitoring drinking and smoking habits and identify the presence of new psychoactive sustances by using high resolution mass spectrometry (HRMS). Raw wastewater samples were solid phase extracted using mixed-mode polymeric phases and analysed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) and HRMS. The daily mass loads of each biomarker were used to back-calculate the use of the selected substances in a population. The profile of cocaine use obtained during a seven-years long monitoring campaign in Milan, showed a marked decrease between 2008 and 2009 (1100-600 g/day of cocaine daily use). On the other hand, a progressive increase of methamphetamine use was observed from 2008 to 2011 (from 10 to 150 g/day). Different patterns of cocaine use were observed according to geographical location and population size in 17 Italian cities. Cocaine use resulted higher in Central Italy and in large cities. On the other side, cannabis use was equally distributed in all the cities investigated. The same approach has been applyed to monitor alchool and smoking habits obtaining specific daily profiles of alcohol and nicotine use in different cities. The smoking habits estimated by wastewater analysis were in line with the Italian prevalence data from epidemiological surveys. The presence of new psycoactive substances in urban wastewater has been screened by HRMS for new drugs such as phenetylamines and cathinones. This study presents a novel and very promising approach, based on mass spectrometry analysis, to provide objective and updated information on human habits in a defined population. It can be considered a complementary tool for epidemiological studies, due to its ability to monitor changing trends in populations, to identify the use of new substances, and to assess

the efficacy of dissuasive campaigns.

185

Occurrence of 940 organic micro-pollutants in environment waters in Hanoi, Vietnam

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This study provides the first widespread information on 940 organic micro-pollutants in environment waters in Hanoi, Vietnam. Surface water (15) and groundwater (18) were collected from 5 rivers/canals and 18 wells, respectively in Hanoi areas from 2011 to 2013. Nine hundred and forty semi-volatile organic compounds (SVOCs) were analyzed by a newly developed comprehensive analytical method using GC-MS, GC-MS-MS and an automated identification and quantification database. The number of detected chemicals in river water ranged from 51-103 (median 82) and 8-30 (median 20) in groundwater. Substances detected in surface water at the highest concentrations were sterols, pharmaceuticals and personal care products (PPCPs) and pesticides, indicating that rivers were heavily polluted with untreated wastewater discharged from domestic sources. Sterols (coprostanol, cholesterol, beta-sitosterol) and fenobucarb were found in 100% samples, followed by PPCPs (caffeine, L-menthol) (90%), whereas permethrin-1 and -2 were only found in urban canals. Concentrations in groundwater were low levels, ranging from 0.67 µg/L to 33 µg/L. However, L-menthol, squalane, diethyltoluamide and caffeine, which were the most frequently detected in the surface water, were also observed in well water, and reaching the maximum values of 0.11 µg/L, 0.21 µg/L, 0.08 µg/L and 5.4 µg/L, respectively. Cholesterol residues (0.05 - 1.3 µg/L) were present in 10 wells and stigmasterol residues (0.05 - 12 µg/L) as well as beta-sitosterol (0.03 - 1.2 µg/L) contaminated 7 wells. Benzyl alcohol was detected in 15 wells with concentration ranged from 0.03 µg/L to 7.1µg/L, which was similar to those observed in surface waters (0.09 - 4.0 µg/L). These results imply that the groundwater is contaminated with wastewater leakage from sewage canals. In addition, calculation concentration ratios of contaminants between the surface water and groundwater using the geometric mean values revealed that contaminants having lower octanol–water partition coefficient values showed higher contribution to the groundwater contamination. Furthermore, since a large number of chemicals detected in surface waters, it is suspected that ground water is contaminated with many chemicals, especially hydrophilic compounds. Consequently, in order to elucidate pollution status of groundwater by organic micro-pollutants, a more detailed survey on hydrophilic chemicals in groundwater is needed.

186

Chiral pharmaceuticals and illicit drugs stereoselective degradation in activated sludge: methodology and results of quantification in microcosm study

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Pharmaceuticals are routinely found within aquatic ecosystems, entering, usually, via wastewater treatment plant (WWTP) effluent, however chirality is rarely considered. Many drugs, including pharmaceuticals and illicits, are chiral i.e. the structures can be present in two or more non-superimposable mirror images, each of which is known as an enantiomer. Each enantiomer, due to its configuration, interacts with biological systems in a unique way. This often results in different adsorption, distribution, metabolic pathways and excretion rates (ADME). Consequently potencies, toxicities and even modes of action may differ between enantiomers. The ADME may also alter when the enantiomers are used in combination due to potential synergistic and/or competitive relationships between the two enantiomers. Post administration, in vivo chiral switching and preferential metabolism often results in an altered enantiomeric fraction being excreted. This new mix of enantiomers is then exposed to biological treatment at WWTPs and ad hoc degradation within aquatic ecosystems, which may continually change the enantiomeric fraction. Chirality, and its implications, are well recognised in drug development with each enantiomer and the mixture being assessed separately. However this phenomenon is not taken into consideration within the European Medicines Agency Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use[1]. This presentation introduces the detection of chiral drugs within the environment and details the analytical technique, chiral LC-MS/MS, used to quantify a range of drugs, including anti-depressants, beta-blockers and illicits, at the enantiomeric level. In addition it will detail the sample preparation techniques used prior to analysis for liquid and solid matrices, e.g. microwave assisted extraction and SPE; illustrating the challenges associated with this, particularly combining the requirements of SPE with chiral-LC. The presented results indicate changes in the enantiomeric fraction during activated sludge treatment. 1. European Medicines Agency, *Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use*, 2006: London, available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003978.pdf.

187

LC-MS/MS Screening for Antibiotics in U.S. Seafood for Consumption

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Aquaculture, the farming of seafood for human consumption, is the fastest growing agricultural sector, providing over 40 percent of edible fish worldwide. This expansion is due to many improvements in the field, such as feed nutrition enhancement and facility conditions, but also due to better control of disease with antibiotics. However, the expanded use of antibiotics in aquaculture also poses significant health risks, one of which is lingering drug residue in the seafood meant for human consumption. We screened for 47 antibiotics in 27 samples of shrimp, salmon, catfish, trout, tilapia, and swai obtained from common grocery stores in SW U.S. Using liquid chromatography/tandem mass spectrometry (LC-MS/MS) in 2 analytical methods, 4 antibiotics were detected above the limits of detection: sulfadimethoxine, ormetoprim, oxytetracycline, and 4-epioxytetracycline. All found concentrations (0.34-8.6 ng/g fresh weight) complied with U.S. regulations. This study provided data on previously unmonitored antibiotics and is the largest screening of antibiotics in U.S. seafood to date. Although results indicate a low risk of drug exposure from seafood consumption, monitoring studies such as the present work are still required to understand and manage potential risks posed by use of antibiotics in aquaculture and in society at large.

188

Investigation of the in vivo uranium-binding protein targets in hepatopancreas and gills of crayfish (Procambarus clarkii) under various aquatic uranium exposure levels

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Uranium (U) is a naturally occurring and/or anthropically (nuclear and military applications) released element in the environment, and its chemical/radiological toxicity is an emerging threat to human beings. As the most stable U species, uranyl ion (UO_2^{2+}) in aerobic media, is able to link oxygen and nitrogen atoms of biomolecules to form non-covalent complexes. However, due to the limited knowledge about *in vivo* U-biomolecules (e.g. protein), it is urgently needed to gain more information for understanding and explaining the toxicity and metabolism of U in organisms under various exposure levels. In our previous works, crayfish (*Procambarus clarkii*) has been used as a biological model to study the U accumulation and identify the potential *in vivo* U-binding proteins after 0, 30, 600 and 4000 µg/L U exposure concentrations [1, 2] representing low-, intermediate- and high-level waterborne exposure. In this study, to have a deeper view of molecular level changes, the cytosolic U-protein complexes were extracted from the gills (organ of entry) and hepatopancreas (organ of storage and detoxication) of crayfish *P. clarkii* and analyzed by non-denaturing one- and two-dimension gel electrophoresis (ND-PAGE and ND-2-DE) coupled to LA-ICP MS and μRPC-ESI MS/MS. The potential U-binding protein targets were identified and compared for the different U-exposure groups. 20 and 6 target proteins likely to bind U were identified in gills and hepatopancreas, respectively. Among those U-haemocyanin and U-ferritin complex were analyzed under all conditions. Furthermore, the U-coeluted haemocyanin and ferritin was quantitatively analyzed and the results showed it had a positive correlation with the aquatic U-exposure concentration.

Acknowledgements: Authors acknowledge the French National Agency (ANR) for the funding of ST MALO - 2010 JCJC 713 1 project supporting this work.

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189

Pesticides in bees, beebread and beeswax: levels and distribution

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Pesticides used in agriculture are nowadays more and more toxic, such as neonicotinoids, pyrethrinoids and carboxamids three pesticides families which are studied here. These pesticides are in particular highly suspected to be involved in bee's abnormally high death rate. When this phenomenon occurs, bees' dead bodies are most often lost and do not allowed a direct analysis on dead bees. The only matrix available to conduct investigations to better understand effects of pesticides on bee health is the beehive and its products. At present, very few data of occurrence and levels of these three molecule families are available in beebread and beeswax, mainly due to lack of analytical methods. Indeed, these matrices represent a real analytical challenge due to their complexity (presence of interfering substances) and the presence of pesticides at very low dose. In this context, the aim of this work was to set up an innovative analytical procedure able to quantify traces of 13 neonicotinoids, pyrethrinoids and carboxamids in bees, beebread and beeswax at low levels (ng/g) in order to evaluate the global view of hive pollution and identify the source of bees' contamination. For this, efficient sample

preparation procedures were developed, based on Quick, Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) extraction for bees and beebread and on solid-liquid extraction using diatomaceous earth as solid support for beeswax. They were followed by a selective and sensitive analysis based on Ultra Performance Liquid Chromatography (UPLC) separation and electrospray tandem mass spectrometry detection (ESI-MSMS). These analytical procedures were validated based on the Guideline validation of analytical procedure 2005 (ICH Norm). For example concerning beebread, extraction recoveries range from 70% to 120%, and limits of Detection (LOD) and Quantification (LOQ) range from 0.014 ng/g to 12 ng/g and from 0.58 ng/g to 40 ng/g respectively. This method was successfully applied to 60 real samples of beebread and beeswax of several areas of France. The final results are presented and discussed. Keywords: pesticides, trace levels, apiarian product contamination, sample preparation

Ecological Consequences of Exposure to Pharmaceuticals: From the Laboratory to the Field (I)

190

Pharmaceuticals in the Environment – the global perspective

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In the past two decades a large number of studies has shown that pharmaceuticals are ubiquitary in the environment in industrialised countries. A concise picture on the prevailing concentrations and potential effects on human and ecosystem health in these countries is still elusive. Therefore, we have started a research project to investigate the state of knowledge on the global relevance of pharmaceuticals in the environment. The project also aims at collecting regional consumption data and trends. We assess the relevance of different emissions pathways and local conditions such as infrastructure, agricultural practice, etc. on the occurence of pharmaceuticals in the environment. The extensive literature review has shown that in 71 countries at least one active ingredient is detected. Globally, 631 active ingredients or transformation products have been detected. Pharmaceuticals in the environment are thus not only an issue of industrialised countries, but a global issue.

191

Risk Based Prioritisation of Pharmaceuticals in the UK

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A range of pharmaceuticals has been detected in the natural environment across the world. While a large amount of data has been published in the past decade on the occurrence, fate and effects of pharmaceuticals, information is still only available for a small proportion of the 4000 pharmaceuticals that are currently in use. Prioritisation approaches are a potentially useful tool to focus monitoring, testing and research resources and to identity those compounds that are likely to pose the greatest risk in a particular situation. In this project, we developed and applied a risk-based prioritization system for pharmaceuticals in use in the UK. The prioritization approach incorporated on usage, metabolism, environmental fate, standard acute and chronic ecotoxicity endpoints and endpoints related to therapeutic models of action. Unlike previous prioritisation exercises, the approach took a holistic view to try to identify pharmaceuticals posing the greatest risk to aquatic and terrestrial species, including wild birds and mammals and humans. The risk posed by the 143 candidate pharmaceuticals (and/or metabolites) were characterised by calculating risk characterisation ratio's (RCRs). Substances were then ranked based on their RCRs. Total of 57 compounds were identified in the top 20 of each of the 9 ranking lists. For many of these substances, no information is available on either the ecotoxicity or occurrence of these substances in the environment. We would advocate that consideration should be given to adding compounds in the top priority list with no or minimal data to future monitoring campaigns and ecotoxicological investigations.

192

Human pharmaceuticals and the Read-Across hypothesis: mammals and fish together at last?

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Pharmaceuticals interact with high affinity to specific molecular targets in the organism, and the drug-target interaction is the key event leading toward the desired therapeutic effect. The evolutionary conservaton of human drug targets in wildlife species suggests that human pharmaceuticals present in the environment may cause pharmacological effects by acting *via* the same targets. The so-called "Read-Across Hypothesis" states that a drug will have an effect in non-target organisms provided that its molecular target is conserved, resulting in a specific pharmacological effect only if blood concentrations are similar to the human therapeutic concentrations. If this hypothesis is correct, it should be possible to predict the likelihood of an effect occurring in fish by using the mammalian data derived from the drug development process, such as pharmacodynamic and pharmacokinetic data, together with genomic and physiological knowledge of the non-target organism. The validation of this hypothesis would represent an important step forward in the large-scale application of predictive approaches based on the maximisation of mammalian data to assess the risk posed to wildlife by pharmaceuticals in the environment. Similar approaches could also be used to prioritise and rank the risk of the 3000 pharmaceuticals on the market, and guide the design of tailored eco-toxicity tests that can efficiently inform the ERA process, avoiding un-necessary tests with low informative power. In this context, we discuss to what extent the Read-Across hypothesis has been validated so far, providing examples of studies performed both at Brunel University and in other laboratories. The applicability of the Read-Across approach, as well as its strengths and limitations are also discussed. Finally, we evaluate the impact that this approach may have on the ERA of pharmaceuticals, since a mere qualitative description of the biological events triggered by chemical exposure would fail to adequately inform regulators and therefore prevent a reliable ERA. The Read-Across approach aims at addressing this issue by answering a fundamental biology question: does the same blood concentration of a given pharmaceutical produce the same pharmacological effect in humans and fish? \n

193

Prioritisation of Pharmaceutical Substances for Monitoring Purposes

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In 2012 UBA introduced a prioritisation list of pharmaceuticals for monitoring purposes in surface water. The list was presented as proposal for institutions being interested or involved in the monitoring process. The list was especially offered to the federal agencies because until now pharmaceuticals are not part of legally regulated monitoring programmes in Germany and Europe. With this prioritisation work the co-operation between federal and state authorities regarding monitoring programmes will be enhanced because federal states asked for support about data on effects, environmental behaviour and consumption of pharmaceuticals to update their own monitoring programmes. Since then the supporting data base for the prioritisation list was updated annually and also used to prioritise pharmaceuticals for groundwater monitoring programmes. From about 2300 active pharmaceutical ingredients marketed in Germay in 2012 approx. 1200 substances were identified as environmentally relevant as starting point for the way of prioritisation. The prioritisation was based on the following parameter: consumption data and their tendency during the last ten years, pharmaceuticals with RQ>1, monitoring data, data about ecotoxicity and behaviour in the environment besides transformation in the body and the environment, expert judgment. Based on the first very positive feedback UBA decided to update and expand this list regularly with pharmaceuticals which arise in regulation with an identified risk to the environment or with increased consumption tendency. Unfortunately, there is a lack of data for high consumption APIs because there are marketed since decades and are thus not covered by the environmental assessment procedure. Especially data about ecotoxicity and environmental behavoiur are missing. This holds true also for most veterinary pharmaceuticals. Consumption data of veterinary substances are not available. Only usage data of antibiotica groups are published recently by the EMA and since 2011 Germany.

194

Defining safe discharge ranges of pharmaceutical compounds in receiving water bodies

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Despite the increasing number of studies concerning the presence of pharmaceutical compounds in the environment, data and knowledge gaps are still numerous. The peculiar physical-chemical properties of these substances contribute to a more complex behaviour in aquatic environments. The main paths for the discharge of these compounds in the environment are wastewater treatment plants (WWTPs), which are inefficient in removing these contaminants by conventional treatments. Most approaches to evaluate the concentration of these substances in WWTPs are based in nationals consumption estimates, which predict overall discharges reasonably well; however, this approach is not suitable when applied to specific locations without knowledge of their characteristics. Furthermore, the removal capacity of conventional wastewater treatments can vary greatly. Thus, aiming to contribute to a better understanding of the presence of pharmaceutical compounds in the environment, a graphical approach to define safe ranges for

discharge of these substances in water bodies has been developed and is discussed in this paper. This work considers the inflows and discharges from WWTPs of five different pharmaceutical compounds: acetaminophen, ibuprofen, carbamazepine, erythromycin and metoprolol. Metabolites, degradation and previous contamination of the water body were not considered, but will be included in future work. The first step was defining ranges in which the target compounds are likely to be discharged from treatment plants in developed countries. For these purposes, measurement data from 31 WWTPs were compiled from literature. Using statistical analysis, minimum, mean and maximum concentration values for the considered compounds were then defined. Afterwards, the removal capacities of three different conventional municipal wastewater treatments were considered to evaluate their removal potentials for these contaminants. Following this, different flows for the receiving water body were analysed to evaluate the dilution factor for different amounts of wastewater released from WWTPs. Lastly, values from chronic ecotoxicity tests were considered to define ranges in which a specific plant could release its effluents without posing any apparent environmental risk. The graphical approach can aid assessing the extent of the environmental exposure to some compounds, strategically defining WWTPs that should be monitored or when attention should be paid to a discharge.

195

How Feasible is Greener Drug Design?

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The issue of pharmaceuticals in the environment (PIE) has received significant coverage in the last decade, both in the academic literature and the wider media. The issue, however, is not a purely recent one. Reviews on PIE have appeared for at least 3 decades, and some have included remarkably prescient content. As early as 1981, a number of pharmaceutical-derived compounds were detected in the River Lee in the United Kingdom. More recent reviews of the literature shows that a wealth of data now exists on PIE and that residues of active pharmaceutical ingredients (API) are widespread, albeit mostly at very low levels, in the aquatic environment. Recent discussions within Europe have resulted in two pharmaceuticals (diclofenac and 17 alpha-ethinylestradiol) and a natural estrogen (17 beta-estradiol) being included within a Watch List so that Europe-wide monitoring data can be obtained about the wider presence of these chemicals in the environment. Consequently, most risk management options are aiming to reduce the environmental burden of PIE by exploring the efficacy of end of pipe tertiary treatment technologies to remove pharmaceuticals and other chemicals from sewage effluent. Parallel to these end of pipe technological solutions, some stakeholders are exploring the feasibility of greener drug design i.e. designing APIs that are biodegradable, non-bioaccumulative and less toxic. This presentation will discuss the challenges faced with designing greener drugs, together with some experimental work validating medium- to high- throughput ecotoxicology assays that will enable the environmental properties of drugs to be studied earlier in drug discovery and development. Results to date, have validated microplate assays with green algae and rotifers against eight human pharmaceuticals for which full OECD test data exist according the 2006 EMA guidance. A microplate assay for anti-infective compounds was also validated with three species of cyanobacteria. These microplate assays predicted the final EC50 and NOEC values for each of the pharmaceuticals obtained in the standard OECD 201 and 211 test guidelines with a high degree of accuracy. They also resolved the relative toxicity of each API. The availability of these validated assays may also assist with the screening and prioritisation of the large number of legacy drug products for which little or no data exist.

Fate and effects of nanomaterials in soil

196

Uptake and elimination kinetics of Ag NPs and ionic Ag in the isopod Porcellionides pruinosus exposed to contaminated soil and food

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Silver nanoparticles (Ag NPs) are widely used, especially due to their bactericidal properties. They may enter the soil through the land application of sewage sludge. However, little is known about their possible risk to soil invertebrates. This study aims at determining the uptake and elimination kinetics of Ag NPs and ionic Ag in the isopod *Porcellionides pruinosus* exposed to contaminated soil and food. Lufa 2.2 soil was spiked at nominal concentrations of 30 and 60 mg Ag/kg, while alder leaves were soaked into Ag NPs or $AgNO_3$ solutions (10 and 20 mg Ag/L). Isopods were exposed individually to contaminated soil or food. Exposure to Ag contaminated soil or food lasted 21 days (uptake phase), and then the isopods were transferred to clean soil or food for another 21 days (elimination phase). Isopods were sacrificed at different time points to determine Ag body burdens. Uptake (K1) and elimination (K2) rate constants were estimated using a one-compartment model, fitted simultaneously to uptake and elimination data. After 21 days exposure, mean Ag body burdens ranged from 90 to 136 µg Ag/g for Ag NPs and from 84 to 164 µg Ag/g for ionic Ag at 30 and 60 mg Ag/kg soil, respectively. K1 did not differ significantly between low and high concentrations of Ag NPs and ionic Ag nor between Ag forms ($X^2_{(1)}$< 3.84). K2 was similar between Ag forms. Bioaccumulation factors (BAF) were 3.0 and 3.6 for Ag NPs and 3.0 and 2.2 for ionic Ag at 30 and 60 mg Ag/kg, respectively. Ag concentration in food was up to 9 times higher for ionic Ag than for Ag NPs (500-800 and 4500-4700 mg/kg for Ag NPs and ionic Ag, respectively). After 21 days exposure, Ag body burdens ranged from 370 to 414 and from 435 to 658 µg Ag/g for Ag NPs and ionic Ag, respectively. K1 differed significantly between low and high concentrations of Ag NPs and ionic Ag, and also between Ag forms ($X^2_{(1)}$> 3.84). Elimination was slower in Ag NPs exposures than for ionic Ag. Bioaccumulation factors were higher for Ag NPs (5.6 and 2.8 for low and high concentrations, respectively) than for ionic Ag (0.4 and 0.3, respectively). Steady state in Ag body burden was not reached in isopods exposed via soil and food after 21 days exposure. When comparing both routes of exposure, dietary exposure resulted in higher body burdens. Moreover, the bioavailability of Ag in soil and food may differ as both mediums present different characteristics.

197

The influence of soil properties on the bioavailability and toxicity of Ag nanoparticles to the earthworm Eisenia fetida

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Metal and metal oxide nanoparticles (NPs) present a series of challenges for terrestrial ecotoxicology. Both chemical transformations of the NPs (e.g. dissolution) and the chemistry of the soil may modify the organism exposure and response in NP–dosed soils. Previous work has shown that soil type influences toxicity of ZnO NPs to earthworms, with soil pH playing a major role. In this study we investigated how soil properties influence the bioavailability and toxicity of silver (Ag) NPs and ionic silver to the earthworm *Eisenia fetida*. Worms were exposed to both a 50nm diameter PVP-coated Ag NP and to an ionic silver reference, silver nitrate (AgNO3). Four soils were used for this study. Soil organic matter (SOM) content ranged from 1.8–16.7% and soil pore water pH from 4.5–8.3. Earthworms were exposed to the nominal concentration range 9 – 5500 mg Ag/kg, with survival and reproduction assessed after 28 and 56 days, respectively. Soil pore waters were extracted from all soils at the beginning (t=0days) and end (t=56days) of the experiment, ultra-filtered (10kDa) and analysed for silver, pH and dissolved organic carbon. Tissue silver concentrations were measured in depurated worms after 28 days of exposure. Based on nominal soil concentrations of silver, the reproduction EC50s in the various soils ranged from 33 - 209 mg Ag/kg for AgNO3 and from 4 - 358 mg Ag/kg for Ag NPs. In the soil with the highest pH and high SOM the EC50 calculated for Ag NPs was lower than that for AgNO3. Furthermore, in two other soils the EC50s calculated for Ag NP and AgNO3 overlapped. This study has shown evidence for NP-specific effects in earthworms exposed in a range of soils. Furthermore, we have seen that soil properties influence the bioavailability and toxicity of silver, whether dosed as a NP or in an ionic form. The results suggest that modelling toxicity in Ag NP-dosed soils needs to consider the dissolution of the NPs to form ionic Ag and the influence of the soil chemistry on the bioavailability and toxicity of both.

198

Preparing soils amended with nano-sized copper oxide, micron-sized copper oxide, and with a copper salt for a nanotoxicity test and results of a 14-day barley growth test

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Toxicity tests determine whether terrestrial organisms such as plants exhibit nanoparticle-specific toxicity when exposed to soluble nanometer-sized metal oxide particles. In these tests, treatments with micrometer-sized particles differentiate effects caused by the material in its non-nanosized form, and metal salt treatments quantify the effects of the metal ions released during particle dissolution. However, differences between, and temporal changes in, soil solution chemistry in the different treatments during tests may obscure nanoparticle-specific effects. In this study on the toxicity of nanosized CuO to plants, we first evaluated temporal changes in Cu^{2+} activity, pH, and dissolved Ca and Mg concentrations in two soils amended with 500 mg/kg Cu as nanometer-sized CuO (nano), micrometer-sized CuO (micron) or $Cu(NO_3)_2$ (salt) over 56 d. The Cu^{2+} activity in the nano-amended

soil was higher than in the micron amended soil and lower than in the salt amended soil for at least the first 14 d. The pH in the nano-amended soil was significantly higher for at least the first 14 d, and dissolved Ca and Mg concentrations significantly lower than in the salt-amended soils for the duration of the test. To minimise differences in soil solution chemistry between the different treatments, salt-amended soils were leached and limed, and all soils were aged for 28 d, prior to the toxicity test. A standard 14 d seedling growth test was then conducted to determine the relationship between Cu^{2+} activity and barley (*Hordeum vulgare* L.) growth. Shoot and root length, and root biomass, in the nano-amended soils were similarly correlated with Cu^{2+} activity as growth in soils amended with non-nanosized Cu sources. Shoot Cu concentrations were similar in all treatments. Barley toxicity in the nano-amended soils was equal to or less than that in soils amended with a similar concentration of the Cu salts. These results contrast with those from some studies conducted in hydroponic systems, and emphasize the importance of conducting nanotoxicity tests in environmentally relevant media.

199

Influence of physico-chemical soil properties on the effect of silver nanoparticles in soils

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The rising use of silver nanoparticles (AgNP) increases the potential for environmental contamination. AgNP will inevitably reach the environment, especially the terrestrial environment. There is a need to gather detailed information about their fate and behaviour within soils. The main goal of this study was to investigate the effects of NM-300K from the OECD Sponsorship Programme on soil microorganisms in five well charaterised soils (RefeSol 01A, 02A, 04A, 03G and 05G) differing in their physico-chemical soil properties. Concentrations of 0.56, 1.67 and 5.0 mg/per kg dry matter soil (dms) for tests with 01A, 02A and 04A and 1.67, 5.0 and 15.0 mg/kg dms for 03G and 05G were observed. Each test was conducted at least twice with 4 replicates per treatment. Standardised test systems were used: C-Transformation (OECD 217) and Ammonium Oxidation (ISO 15685). All experiments met the validity criteria. The C-transformation test was less sensitive. Only in one test (01A) an EC_{50} of 5.6 mg/kg dms was calculated. In contrast nitrifying bacteria (ISO 15685), were extremely sensitive to 28 days exposure to AgNP. In each test a dose-response relationship was found. Considering the EC_{50} values the highest toxicity occurred in the tests with 04A (1.2 mg/kg dm soil), whereas the lowest toxicity was observed for 03G (28.4 mg/kg dm). A comparison of the toxicity of AgNP with the physico-chemical soil properties showed a decreasing toxicity with increasing clay content (04A: 4%; 03G: 27%). The pH value also appears to play an important role in soils in relation to the toxicity of AgNP. The strongest toxicity was found at pH values of 5.03 (04A), 5.30 (01A) and 4.59 (05G). Here is evident that the toxicity at a pH of 4.59 (05G) was lower than at a pH of 5.30 or 5.03, while the clay content and C_{org} of the 05G were significantly higher than in the soils with a higher pH value (04A, 01A), which may reduce the influence of the low pH. Based on the present results a dependence of the toxicity of AgNP on the C_{org} content could not be shown. The study shows that toxicity of AgNP cannot be attributed to only one soil parameter. The same soil parameters as for conventional chemicals influence toxicity, e.g. for metals, soil pH and parameter affecting sorption behavior are also important. Therefore, concerning ecotoxicity studies the recommendations in the test guidelines on soils are considered to be applicable to achieve maximum effect and to simulate worst case scenarios.

200

Combining physico- chemistry and microbial ecotoxicology to assess the impact of TiO2 nanoparticles in 6 agricultural soils

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Titanium dioxide nanoparticles (TiO_2-NP) are one of the most widely used nanomaterials to date. TiO_2-NP are chronically released into the environment, especially in agricultural soil through sewage-sludge application as fertilizers. Information regarding the impact of a realistic concentration of NP on soil organisms is currently limited and the importance of soil characteristics is often neglected in ecological risk assessment. As yet, there are few data on transport of NP through soils, and hence characterization of NP behavior and associated potential bioavailability remains to be elucidated. Extrapolation of results from one contaminated soil to another is difficult because of the great variability in soil composition and structure. Thus establishing the relationships between soil properties and (i) NP behavior and (ii) ecotoxicity is urgently needed in order to evaluate the vulnerability of soils, and especially of microorganisms to NP. In this study using 6 contrasted soils, we characterized the size and surface charge of TiO_2-NP in soil solutions and we studied their transport in a soil column experiment. In addition, we assessed the response of soil microorganisms in a microcosm experiment, where the 6 soils were exposed for 90 days to a realistic concentration or an accidental spiking of TiO_2-NP (1 and 500 $mg.kg^{-1}$ dry soil). Soil respiration and microbial activities of the nitrogen cycle (nitrification and denitrification) were measured, in order to assess the effect of TiO_2-NP on soil functioning. Microbial abundance of bacteria, nitrifying and denitrifying microbial communities were also determined by quantitative PCR targeting *16S rRNA* and functional genes (*amoA*, *nirK*, *nirS*). We found that soil properties, especially pH and ionic strength, can influence the aggregated size and surface charge of TiO_2-NP. In the column experiment, we observed that TiO_2-NP exhibited very different transport capacities depending on the clay content of the soil. In most soils, TiO_2-NP had no impact on microbial communities, except in a silty-clay soil where microbial activities and abundances were significantly lowered, even with the realistic NP concentration. Altogether, our results demonstrate the importance of assessing the impact of a realistic concentration of NP in different soils and the relevance of NP characterization in realistic conditions such as soil solutions, in order to get a better assessment of the impact of NP on soil functioning.

201

Diversity, activity and abundance of microbial functional groups as descriptors of soil disturbance: Example of contamination with TiO2 nanoparticles

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According to the conceptual model of Zak et al. (1994), total biodiversity relates to genetic, taxonomic and functional diversity. However, in most studies dealing with soil microbial biodiversity, functional diversity is often neglected, despite the crucial information that can be given about the response of soil to environmental stressors. In soil, key functions related to fertility are carried out by different microbial functional groups that may respond differently to pollutants due to the diverse levels of functional redundancy among these groups. Here, we assessed the impact of an emergent pollutant, titanium dioxide nanoparticles (TiO_2-NP), on soil functioning using the diversity, the abundances and the activities of different microbial groups as descriptors of the response to such disturbance. To evaluate the impact of TiO_2-NP, a silty-clay soil was exposed for 90 days to an environmentally relevant concentration or to an accidental spiking of TiO_2-NP (1 and 500 $mg.kg^{-1}$ dry soil, respectively) in microcosms. The effects on microbial activity (Carbon and Nitrogen cycle) were assessed by substrate-induced respiration (SIR), potential nitrification and denitrification rates. Modifications in bacterial abundance were evaluated by quantitative PCR targeting *16S* rRNA bacterial gene and functional gene of nitrification (*amoA*) and denitrification (*nirK* and *nirS*). We used the Illumina sequencing platform (MiSeq) to explore the effects of TiO_2-NP on the diversity of bacterial and archaeal communities and of two functional groups: the nitrifiers, ammonia-oxidizing bacteria and archaea. We will present the results showing that TiO_2-NP can alter 3 crucial microbial functions in soil, even after a contamination with a realistic concentration (1 $mg.kg^{-1}$). The nitrification which is performed by the AOA and AOB groups exhibiting a low functional redundancy compared to the denitrifiers, appeared to be more affected by TiO_2-NP than the latters. Using path analysis, we will show that the mortality among ammonia-oxidizing archaea group had cascading effects on nitrification and denitrification activities. We will discuss the unexplained variance in this model in the light of the diversity results on functional genes. In conclusion the importance of studying functional diversity in ecotoxicological studies, to have a better assessment of the impact of a pollutant on soil functioning will be highlighted.

Bioavailablity and effects of metals and metal mixtures (I)

202

Interactions between copper and salinity in the teleost fish sheepshead minnow: a multi-stressor approach

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Despite having been extensively studied for many decades, metal contamination remains an unresolved environmental problem, as metals display toxic effects on the aquatic biota at environmentally realistic concentrations. It has been long accepted that their bioavailability is strongly affected by chemical factors, such as water hardness and organic matter content, and this knowledge has led to the development of Biotic Ligand Models (BLMs) aiming at predicting metal toxicity on the basis of their speciation and interaction with abiotic factors. However, one side effect of this research pathway has been a general tendency to address metal toxicity mainly from a chemical perspective, partly overlooking the physiological mechanisms underlying their toxicity. Considering copper as a case study, BLMs account for the effects of water chemistry on copper speciation, but do not consider its influence on fish physiology, whereas we argue that chemistry affects copper toxicity by affecting both its speciation and fish osmoregulatory physiology. Applying this perspective, we investigated the interaction between copper and salinity by mimicking the salinity changes taking place in estuaries, which are environments where the combination of anthropogenic impacts and fluctuating

abiotic factors represents the ultimate challenge for toxicity modelling. Since copper toxicity is mainly a consequence of osmotic disruption, we hypothesize that copper exposed fish are more sensitive to osmotic stresses, as copper affects their osmoregulatory system by interacting with some osmotic effector proteins. Among these effectors, we suggest that a common factor linking the physiological responses to both copper exposure and salinity changes is the enzyme carbonic anhydrase (CA), a copper target with salinity-dependant activity. Two *in vivo* studies were performed on the euryhaline fish sheepshead minnow, which was exposed to 10 and 100 μg/L in the first study and 32, 100 and 320 μg/L copper in the second study for, respectively 9 and 21 days. After copper exposure in either freshwater or artificially prepared saltwater (20ppt), a salinity transition was performed. Measurements of gene expression levels of CA in samples of gills and intestine showed that this enzyme significantly responded to copper, making of it a candidate probe to explore the complex response patterns to environmental as well as chemical stressors, such as osmotic stress and metal pollution.

203

Copper toxicity in mussels: do salinity, organic matter and population history matter?

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Salinity and dissolved organic carbon (DOC) are two abiotic variables that can alter Cu toxicity to marine organisms due to complexation and speciation. In this study the sublethal effects of prolonged Cu exposure on juvenile transformed *Mytilus edulis* were assessed under different conditions of salinity and DOC in 2 populations (North Sea (NS) and Bothnian Sea (BS)). First, separate experiments were set up for each population. Mussels acclimated to 5 salinities were exposed for 2 weeks to 18 different salinity/DOC/Cu combinations, according to a central composite design. At the end of the exposure the clearance rate (CR), oxygen consumption (VO_2) and condition index (CI) were measured. Next, both populations were simultaneously assessed. Now DOC was not varied and salinity was identical for the two populations. For the NS population, no effect of salinity on the CR was observed. An increase in DOC slightly increased the control CR, but the interaction with Cu was marginal. No DOC effect on CR was observed in the BS population. However, salinity had a strong positive effect, increasing control CR without altering Cu toxicity. In the NS population, VO_2 slightly increased with increasing DOC without interaction with Cu. An increase in salinity increased control VO_2 in NS mussels, but in combination with Cu this resulted in a faster VO_2 decline. The effect of salinity was more pronounced in the BS population: an increase in salinity increased the control VO_2 without interaction with Cu. An increase in DOC decreased the control VO_2 with little influence on Cu toxicity in the BS mussels. Salinity and DOC did not affect the CI in either population. The simultaneous experiment yielded similar results. *M. edulis* from the BS population live near the edge of the salinity tolerance range. Increasing metabolic activity with increasing salinity demonstrates that under natural conditions this population experiences salinity stress. Nevertheless, when corrected for this change in baseline metabolism, BS mussels are as (or less) sensitive than NS mussels. Contrary to what was expected based on speciation and complexation chemistry, an increase in DOC or salinity did not, or only slightly, decrease the sensitivity to dissolved Cu. Therefore it seems that free Cu ions are not the only toxic Cu species and Cu-DOC complexes might be available for uptake by the mussel. This indicates that the current BLM concept is not applicable to *M. edulis* for the measured endpoints.

204

Protectiveness of copper aquatic life criteria/guidelines against olfactory impairment in fish: An international comparison

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Several laboratory studies have demonstrated that short-term exposures to low Cu concentrations can cause olfactory and behavioral effects in Pacific salmon and trout, which may be indicative of adverse effects on olfactory-mediated behaviors in the field (e.g., ability to avoid predators). Concerns have been expressed in North America and Europe that current water quality criteria/guidelines for Cu are not protective against olfactory impairment. Importantly, such concerns are founded on simple comparisons that do not properly account for how water chemistry influences the bioavailability of Cu. We have previously demonstrated that the USEPA's biotic ligand model (BLM)-based aquatic life criteria for Cu are protective against olfactory impairment over a wide range of fresh waters. We further evaluated the influence of Cu on olfactory impairment in additional fish species by critically reviewing recently published studies and evaluated whether Cu criteria/guidelines from states and other countries/jurisdictions (e.g., Canada and its provinces, European Union) are protective against olfactory impairment. Most countries and jurisdictions still apply hardness-based Cu criteria. Overall, 223 different olfactory threshold-to-hardness-based-guideline ratios were derived from the compiled data. The ratio was

205

Deriving waterborne safe tresholds for Pb in European freshwaters

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In the REACH framework, Pb specific information on environmental toxicity and on environmental exposure/fate for key environmental compartments (water, sediment, soil) was compiled in order to assess the potential environmental risks related to the production and use of Pb in the European Union. The methodologies and concepts used in the present study represent the state-of-the-art about the Pb specific aspects related to incorporation of bioavailability and large dataset handling. The proposed methodology encompasses the development of an extensive quality screened database containing 173 individual chronic toxicity data for 25 different freshwater species and takes the effect of 1) Pb precipitation and 2) Pb speciation/competition on Pb toxicity fully into account by the use of newly developed Pb-translator and chronic Biotic Ligand Models (BLM) for 3 different trophic levels (algae, invertebrates and fish). As such the freshwater Pb effects database can be easily normalised towards the geochemical conditions prevailing in the EU surface water under scrutiny, and revealed that the safe toxic threshold for the freshwater environment (HC5-50) is expected to vary between 8.8 and 29.6 µg dissolved Pb/L. The statistical best fitting approaches that were used for the derivation of the freshwater HC5-50 from the species sensitivity distributions will be further discussed. Finally, the conservatism and remaining uncertainty considered for the final HC5-50 setting derivation will be highlighted and a way forward in order to reduce the uncertainties associated with metal risk assessments in general will be proposed.

206

Trivalent chromium chemistry: when considered, makes it more toxic than the hexavalent chromium in algal toxicity test

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In surface waters, chromium occurs mainly in two oxidation states, namely Cr(III) and Cr(VI), and there is a general agreement that Cr(III) is less toxic than Cr(VI) for aquatic organisms. However, an increasing number of studies provides evidence that Cr(III) may also be of concern and that, in some cases, its toxicity may have been underestimated. In this study, the temporal variability of filterable (0.22 μm) Cr concentrations in a standard algal culture medium (ISO 8692) was followed over 72h (the typical duration of the corresponding toxicity test). In parallel, the toxicity of three trivalent Cr salts (sulphate, chloride and nitrate forms) and one hexavalent Cr salt (potassium dichromate) to the alga *Pseudokirchneriella subcapitata* was also studied. Over 72 hours, chromium concentration was stable in solutions amended with Cr(VI), but decreased markedly (with losses up to 90% of the initial Cr levels) in solutions containing Cr(III). The nominal EC50 values were similar for both Cr redox forms. However, when the decrease of concentration was considered by calculating the time weighted mean, the Cr(III) EC50 became lower than those for Cr(VI). These results suggest that, provided Cr(III) behavior is properly considered, trivalent Cr may actually be more toxic than Cr(VI); at least in standard laboratory media.

207

A dynamic Biotic Ligand Model predicting Ni toxicity to Enchytraeus crypticus

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Biotic ligand models (BLMs) have been shown to effectively predict metal toxicity to organisms exposed to (soil) solutions of varying composition. Until now only BLMs developed at a fixed time point are available, triggering the question of how to extrapolate such predictions to a dynamic environment with time-variable exposure. In this study Ni-BLMs were therefore developed for *Enchytraeus crypticus* at different exposure times (7, 10, and 14 day) to examine the applicability of the BLM approach for predicting Ni toxicity over time. Results showed that the same cations (Ca^{2+}, Mg^{2+} and Na^{+}) significantly alleviated Ni toxicity at different time points but to different degrees. The stability binding constants of Ni and the competitive cations decreased with increasing exposure time, but the fraction of biotic ligands occupied by Ni causing 50% mortality remained constant in the time course. A novel dynamic BLM model for predicting time-variable toxicity of Ni was developed, which accurately predicted Ni toxicity at different water chemistries and exposure times. Our findings indicate that time needs to be considered as a factor in modelling metal toxicity and that the existing acute BLM may not be applicable for predicting metal toxicity under chronic exposure. More research is needed to investigate the exact mechanisms behind the

time-related changes of BLM binding constants.

Modelling of chemical fate and exposure in a regulatory context (I)

208

Simulation of pharmaceuticals in the environment

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Indirect human exposure to high-volume drugs via wastewater to rivers and via sewage sludge to agricultural soils and bioaccumulation in fish and crops was simulated with coupled mathematical models. Most drugs are ionizable, often zwitterionic, and the models were modified to be valid for such compounds [1,2]. Use data in Denmark from medstat.dk corrected for metabolism in human body were selected as input data. Concentrations in rivers were calculated with the common dilution factor 10. The bioconcentration factor fish was estimated with a dynamic cell model based on the Fick-Nernst-Planck equation, including adsorption to proteins. A validated simulation model [3] considering transfer of water and solutes from soil to plant and leaching to groundwater was adopted and reprogrammed for ionic compounds. Highest human usage of antibiotics in Demark is for erythromycin, tetracycline, ciprofloxacin, doxycycline and trimethoprim. Most antibiotics adsorb strongly to sewage sludge, with resulting concentrations > 1 mg/kg. Sewage sludge application as soil amendment leads to calculated concentrations of some antibiotics in top soil > 10 microg per kg. The results confirm that some antibiotics show good plant uptake. The comparison of measured data to simulation results at all stages (effluent water, sewage sludge, soil, plants) was satisfying. Summarized, the major conclusions are that this simulation system, adopted to ionizable compounds, can be used to predict emissions from wastewater, residues in soil and plant uptake of pharmaceuticals (and pesticides); that dietary exposure to human antibiotics is higher with field crops than with (river) fish; that uncertainties and variations (also of experimental data) are high. [1] Trapp S, Franco A, Mackay D. 2010. Activity-based concept for transport and partitioning of ionizing organics. Environ Sci Technol 44, 6123-6129 [2] Franco A, Trapp S. 2008. Estimation of the soil-water partition coefficient normalized to organic carbon for ionizable organic chemicals. Environ. Toxicol. Chem. 27:1995-2004 [3] Trapp S, Eggen T. 2013. Simulation of the plant uptake of organophosphates and other emerging pollutants for greenhouse experiments and field conditions. Environ Sci Pollut Res 20:4018–4029

209

Persistent organic pollutants – Analysis of sources, spatial distribution, and reservoirs in Switzerland

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Persistent organic pollutants (POPs) are ubiquitous environmental contaminants because of their appreciable mobility and resistance to degradation by environmental transformation processes. To understand the distribution and transport of POPs in the environment, multimedia fate models are useful. Within the project SwissPOP we developed two models to study sources, reservoirs and sinks of POPs in Switzerland. Main questions in this context are e.g. which environmental compartments represent currently the main reservoirs of POPs in Switzerland and how do these reservoirs behave in the future, how and when are concentrations of POPs in the environment affected by regulatory measures, how relevant are secondary sources compared to primary sources now and in the future, which effect have altitude and distance to the sources on the environmental levels of the chemicals, and are the high Alps relevant in the environmental cycling of POPs. First we developed a one-region multimedia fate model that consists of four well-mixed compartments (air, soil, freshwater and sediment) and describes the distribution of the chemicals between these compartments. Since most measurement data of POPs in Switzerland are available for polychlorinated biphenyls (PCBs), we used the model runs for PCBs to validate our model. The modelled results for PCBs show very good agreement with the absolute concentrations of measurements in soil, air, and water. Also the pattern of individual PCB congeners calculated with our model is consistent with field data, indicating that the model covers all important exchange processes between the compartments. In a second stage, we develop an extended ten-region multimedia fate model that tracks the mass balance of POPs in Switzerland, subdivided in ten regions, where each region is composed of six well-mixed compartments (troposphere, convective boundary layer, nocturnal boundary layer, freshwater, sediment, and soil). Chemicals are emitted to air, and can be distributed within the region or transported to other regions by wind and water. The parameterization of the atmospheric circulation in the model was done with weather forcast data for Switzerland. The model results can be used to confirm measured concentrations, but also to predict concentrations under different regulations scenarios in the future. It will therefore become a very useful tool for science and regulatory authorities.

210

Unravelling the relationship between body mass index and persistent organic pollutant concentrations in humans

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Because of their hydrophobicity, persistent organic pollutants (POPs) associate with the lipid compartments in the human body. Individuals vary widely in terms of their lipid content. Human biomonitoring studies often observe that the measured concentrations of POPs are statistically associated with the body mass index (BMI). However, the results are inconsistent, showing negative, positive or no associations. These divergent relationships have been hypothesised to be the result of variable pharmacokinetics of POPs in lean and obese individuals during times of increasing and decreasing exposure. To test the plausibility of that hypothesis, we used the CoZMoMan model to calculate time-variant concentrations of PCB-153 for different human birth cohorts born throughout the 20th century based on time-variant historical dietary contamination. For each cohort, multiple calculations representing five different BMI classes were performed, using generic, but realistic assumptions about weight and lipid gain during the adult life span. The results of cross-sectional human biomonitoring studies were then simulated by “sampling” a cross-section of the simulated cohorts in different years. Using historical data on the BMI distribution in the US, concentrations calculated for different cohorts and BMI classes were averaged to predict population level relationships between POP concentrations and BMI and age that can be expected to be observed in biomonitoring studies conducted during different sampling years. The simulated relationships showed a dependence on both age and sampling year. Whereas the model predicts clearly negative associations between PCP-153 concentrations and BMI for all birth cohorts in monitoring studies conducted in the 20th century, future biomonitoring studies are predicted to reveal more complex relationships. The model results suggest that different age cohorts can display different concentration - BMI relationships. As a result, the PCB-153 concentration - BMI relationship expected in human biomonitoring studies will depend on the sampling year, and on the ranges of age and BMI classes that are represented in the sampled population. Mechanistic models of human bioaccumulation can aid in unravelling the complex relationship between age, BMI and POP concentrations. Doing so is imperative in any effort to understand the potential obesogenic effect of POPs.

211

Influence of climate change on the Arctic Contamination Potential

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Using the Danish Eulerian Hemispheric Model (DEHM) we have calculated the Arctic Contamination Potential (ACP). ACP is defined as the sum of masses in the arctic surface compartments (soil, vegetation, snow and water) at the end of a ten year simulated period normalised either with the total mass within the model domain of with the total amount emitted into the atmosphere during the ten year simulation. In this study we use the emission normalized ACP termed eACP. We have calculated the eACP for the physical-chemical phase space spanned by compounds with log K_{oa} between 3 and 12 and log K_{aw} between -4 and 3 and for each point in this phase space grid we have included a perfectly persistent compound in the model. DEHM is a 3-D atmospheric chemistry-transport model modelling the atmospheric transport of four chemical groups: a group with SOx-NOx-VOC-ozone chemistry, a group with primary particulates, a mercury chemistry group, and finally a group with Persistent Organic Pollutants with 2-d surface compartments (soil, vegetation, ocean water and a dynamic temporal snow cover) with inter-compartmental mass exchange process parameterizations. The model domain covers the Northern Hemisphere and thus includes all important source areas for the Arctic. The spatial horizontal resolution of the model system in this work is 150km x 150km and the model includes 20 vertical levels up to approximately 15km above the surface. The model system was run with meteorology obtain from ECHAM5/MPI-OM (SRES A1B scenario) for two decades: 1990-1999 and 2090-2099. Highest potential (12%) for reaching the Arctic surface compartments for the 1990s is seen for compounds with low log Koa and low log Kaw values. These are relative water soluble compounds referred to as “swimmers”. For the 2090s, the overall pattern of the ACP phase space is similar to the pattern for the 1990s. ACP is generally larger for the 2090s than for the 1990s, with a maximum of 15%.

212

The Dirt on Predicting Veterinary Drug Concentrations in a Canadian Context From A Regulatory Prespective

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A Canadian regulatory framework has been developed specifically for active pharmaceutical ingredients (APIs) regulated by the *Food and Drugs Act* (F&DA) to assess their potential environmental effects in soils, sediments and surface waters. This regulatory framework was designed to harmonize with the drug development process as well as approaches to environmental assessment in other jurisdictions. APIs in veterinary drugs administered to food animals primarily enter ecosystems

through the application of livestock waste to agricultural land and a means to predict environmental concentrations (PECs) of APIs under various scenarios and in different compartments is required as part of a directed testing approach. The complexity of reducing the uncertainty in the calculation of a predicted environmental concentration of veterinary APIs in soil (PECsoil) and variability in agricultural practices can be a challenge for regulatory authorities. A science-based and transparent methodology to calculate PECsoils, based on an approach used in Europe under VICH, has been established for Canadian scenarios. Default values and livestock categories have been developed which are representative of typical confined animal husbandry and agricultural practices in in Canada and will be used to identify new veterinary APIs that require terrestrial ecotoxicity and fate testing. Adaptation of the European PECsoil calculation to Canadian agricultural practices required the addition and/or adaptation of livestock categories based on an analysis of Canadian production practices and labels for currently registered veterinary drugs. Default intake rates to estimate doses of APIs administered in feed and water were also established. To validate the conceptual basis of this approach PECsoil values for a subset of currently used APIs are compared to data from field experiments and monitoring studies. Measured environmental concentrations of APIs, including those commonly used as feed additives, in agricultural fields and pastures that have received applications of manure from pig, poultry or cattle agricultural facilities will be discussed. A comparison to PECsoil values for APIs generated with approaches used in the United States and the European Union will also be presented. Finally this talk will explore how this model might be utilized to better direct the testing requirements for new veterinary APIs within the proposed regulatory framework.

213

Multi-dimensional modelling of leaching for ridge-furrow systems

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The assessment of exposure and leaching of pesticides in soil is most often done with one-dimensional (1-D) models. However, for inhomogeneous soil surface applications (e.g. ridged cultivation systems) these 1-D models are inappropiate, as the geometry plays a significant role in the system characterization, and thus also in the resulting leaching assessment. In this study, two scenarios using ridged systems are investigated and analysed. The first considers a standard spray application to a surface consisting of a furrow and ridge. The second considers the same geometry but with an in-row applied pesticide which is placed at a certain depth below the peak of the ridge. Simulations were carried out using the multidimensional flow and transport model HYDRUS 2D/3D and a 2-D simulation domain. In all calculations, inhomogeneous infiltration was implemented. Moreover, inhomogeneous chemical loading was considered via i) variable pesticide loading at the surface, and ii) in-row application where the applied pesticide is located in a band (10 by 10 cm) which is centered 10 cm below the ridge peak. The soil parameters and the climate data were taken from the FOCUS Hamburg groundwater scenario. For the sake of simplicity an uncropped situation was considered. The pesticide used was carbofuran. The simulation period was set to allow for practically complete breakthrough of leached solutes at the evaluation depth (1 m). The concentration distributions for inhomogeneous spray surface application and in-row application differ strongly. This is on the one hand the result of the inhomogeneous boundary conditions applied to each scenario. On the other hand the difference in leaching is caused by the difference in travelling distance between ridge and furrow towards the target depth of 1 m. This results in largely different ratios of mass leached/applied for spray surface and for in-row application. Spatially inhomogeneous water flow and pesticide application requires a multi-dimensional representation to obtain realistic leaching results. By definition 1-D representations cannot reproduce such transitional behaviour. They cannot consider the spatial variability in leaching caused by the surface geometry, rather they can only assume either complete or no mixing, which will result in either under- or overestimation of the true leaching.

Advancements in life cycle impact assessment and footprint method development (I)

214

Impact Pathway Characterisation to evaluate sustainability performance of products by addressing six Safeguard Subjects

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To assess products and services with regard to their potential impacts on sustainability are “of high priority for international, national and local administration and enterprises” (Traverso et al. 2012, p.680). To evaluate the sustainability performance of a product can be done by life cycle based assessment, integrating environmental, social and economic aspects. The integrated assessment method addresses six safeguard subjects: the three (1) human health, (2) ecosystem quality and (3) resource availability commonly used and broadly accepted in LCA as well as the other three (4) social justice, (5) financial stability and (6) man-made environment (Scheumann et al. 2013). Each safeguard subject has one or more endpoint category indicators. A possible solution for addressing the defined endpoints is the development of possible impact pathways emanating from the final endpoint category indicator over midpoint impact indicators down to the inventory data. Data on the inventory level are then assigned to an endpoint category representing sustainability aspects of concern on environmental, social or economic mechanism. Several impact pathways are derived to show exemplarily applicability for the case of fertilizers. The safeguard subject *social justice* is connected to the endpoint category indicator status of working environment, among others. Related midpoint indicators are e.g. income inequality, observing labour rights, occupational status and economic prosperity. On the inventory level time based contracts, typical for seasonal workers, show to have an impact on the employees, which are quantified at the midpoint impact category occupational status as well as at the endpoint category status of working environment. In addition, the short time contracts may cause stress at the level of the employees which leads to an effect on occupational status at the midpoint and damage to human health at the endpoint level. However, time contracts might be a way to regulate the company’s day-to-day routine and may support future development of the enterprise. In that case, the impact indicator economic prosperity has a linkage to the safeguard subject financial stability via the endpoint microeconomic performance. This integrated proposal offers a different approach to evaluate possible impacts of products via LCSA. Still, the environmental impacts related to emissions from e.g. production of fertilizer are better accounted in comparison to the relative reference points of the cause-effect modelling e.g. to address social justice. Scheumann, R. et al., 2013. A new indicator framework for LCSA considering safeguard subjects. In *Proceedings of the SETAC Europe 23rd Annual Meeting*. Glasgow: SETAC Europe. Traverso, M. et al., 2012. Life Cycle Sustainability Dashboard. *Journal of Industrial Ecology*, 16(5), pp.680–688. Available at: http://dx.doi.org/10.1111/j.1530-9290.2012.00497.x.

215

Proposal for a new normalization reference in LCA based on “safe operating space”: presentation of framework and global factors at midpoint level

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Planetary boundaries have been suggested for a range of environmental impacts, such as climate change, eutrophying nutrients and land use. The boundaries demarcate the safe operating space of humanity: Staying within the space ensures environmental sustainability, while exceeding it risks pushing ecosystems into alternative regimes, leading to adverse effects for humanity. Planetary boundaries can be applied as policy targets. To promote a societal development in the direction of these targets, an indicator system is needed that measures the fraction of the safe operating space that a given activity occupies. We propose that such an indicator system can be applied in life cycle assessment (LCA) by integrating planetary boundaries via the normalization step. We present the framework of integration, a literature review of quantified boundaries and resulting normatively consistent global average normalization factors in units compatible with characterized results at midpoint level in LCA. Our suggested framework allows expressing normalized results in units of “sustainable person years”. Normalization factors are derived by dividing the safe operating space by the global population. The proposed normalization factors were compared with existing normalization factors that are based on global impacts currently taking place. The impact categories climate change, land use and terrestrial acidification were found to have their safe operating space exceeded on average globally, while the opposite was true for the remaining six categories assessed. Additional research is needed with respect to spatial differentiation since the derived global normalization factors have reduced environmental relevance for impact categories operating at the regional or local scale. Nevertheless the developed normalization factors represent an important first step in enabling LCA to help guiding society in the direction of staying within the safe operating space.

216

Spatial differentiation of LCI datasets: implication of new characterisation methods and necessity of a finer data collection to reduce LCIA results uncertainties - The example of EEE products

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The electric and electronic (EEE) sector is a globalized market. Industries are spread worldwide, same products can be manufactured in various countries leading to different environmental impacts. Moreover, some newly developed characterisation methods include spatial differentiation of their characterisation factors. Therefore, it is important to keep environmental data concerning EEE products up to date in order to provide to life cycle assessment users the most reliable data and reduce the LCIA results uncertainties. Besides, that strong need of updating LCI datasets is facing the difficulty to access to data on new technologies. Indeed, the manufacturing processes are often trade secrets: industrials are not willing to reveal them because of the highly-competitive market and the risks associated. The EEE sector also has a very complex supply chain, involving many

factories from various countries making an overall vision difficult. Because of that some data have to be extrapolated considering available scientific and industrial data sources, and some hypotheses have to be made. These hypotheses induce uncertainties that must be qualified and/or quantified. This presentation aims at qualifying and quantifying the uncertainties on LCIA results led by both the spatial differentiation of LCI datasets and of the characterisation methods, then determining what the improvement of LCI dataset and LCA data collection must be to achieve coherent LCA results and interpretations. A generic silicon semiconductor component will be used as an example through the presentation.

217

Connecting damage-oriented and resource-oriented environmental footprints

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Two main schools of thought can be distinguished in the field of life cycle impact assessment. One school uses very simple resource-oriented methods as approximations of environmental impact while the other school uses sophisticated models to determine the impact of as many environmental interventions as possible. An advantage of the resource-based indicators is that they are relatively easy to calculate and communicate. It is unclear however whether these simple indicators are truly representative of the total environmental burden. In this study we determine whether the simple resource based footprints are good approximations of the environmental damage. Therefore we have used a multiple linear regression model in which the environmental damage (to humans, ecosystems and resource availability) was predicted based on four resource use indicators (land, non-renewable energy, water and material). The environmental damage for 959 commodities was determined by using an updated version of the ReCiPe methodology. Data on the environmental interventions of these commodities was taken from the ecoinvent database (version 3.0). Our results showed strong correlations between the two approaches (R^2 values were between 0.79 and 0.94). The most important predictors of environmental damage varied per area of protection and (to a lesser extent) per cultural perspective. However, non-renewable energy demand was found to be a good predictor for all three areas of protection. For human health, material use was found to be important as well, while for ecosystems taking into account land use was of added value. Our results indicate that policies that are aimed towards reducing one resource footprint (for example, energy use) are *ceteris paribus* likely to also reduce the overall environmental damage substantially.

218

The EU Organisation Environmental Footprint Sector Rules (OEFSR) Retailers pilot testing

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On April 9th 2013, the European Commission published: "Communication from the Commission to the European Parliament and the Council: Building the Single Market for Green Products, facilitating better information on the environmental performance of products and organisations". An open call for volunteers was announced by the European Commission for the Product Environmental Footprint (PEF) and the Organisation Environmental Footprint (OEF), inviting companies, industrial and stakeholder organisations in the EU to participate in the development of product-group specific and sector-specific rules. A group of public and private organisations has been selected by the European Commission to develop the guidance for surveying and reporting environmental impacts in the European retail sector. The Technical Secretariat which is responsible for developing the OEF sector rules in two years (official launch in November 2013), is composed by three retailers: Carrefour SA, Colruyt Group and Oxylane Group (Decathlon); three public agencies: Environment Agency Austria (EAA), French Environment and Energy Management Agency (ADEME) and Italian National agency for New Technologies, Energy and Sustainable Economic Development (ENEA); one non-governmental organization: Global 2000; and one Life Cycle Assessment (LCA) consultant: Quantis. This pilot will test how approaches such as Product Environmental Footprint Category Rules (PEFCRs) and the "Chain OEF" (approach covering the indirect/upstream part of the value chain) will interact with or benefit the proposed OEFSR. The project, challenges and expected results and benefits of this pilot test will be presented highlighting feedback in reference to specific modelling issues related to the application of LCA to a sector as vast as the retail sector such as defining system boundaries (e.g., direct, as well as upstream and downstream indirect contributions) and choosing life cycle impact assessment methods (e.g. which indicators are relevant, which weighting scheme to use). These points also include the issue pertaining to consistency with the product approach for a sector as interdisciplinary as the retail sector.

Risk assessment of chemical mixtures: strategies, bottlenecks, and the steps ahead (I)

219

Towards a common conceptual framework for chemical footprint bridging Risk Assessment and Life Cycle Assessment: Short review and way forward

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Several studies have been presented recently, applying the chemical footprint (ChF) concept trying to address a variety of questions, often, but not always, to aggregate pollution of many chemicals to one or a few indicators. Furthermore, the possiblity to link chemical pollution to the concept of planetary boundaries, e.g. through the ChF concept, has also been discussed in recent publications. While the planetary boundary concept is pointed out as very difficult for chemical pollution, because of its local or regional nature, there is a need for an integrated chemical assessment and management approach on the regional and global level. This paper provides a short review and conceptual analysis regarding ChF, and suggests a way forward towards a common science based Conceptual Framework for Chemical Footprinting methods, bridging Risk Assessment (RA) and Life Cycle Assessment (LCA) science and methods. Although varying, the approaches reviewed typically are rooted in the knowledge basis of both RA and LCA. Questions for further elaboration are, e.g.: (a) Is a ChF assigned to an object in the technosphere: point source, value chain, sector, or the whole economy, and if so, on what scale (Sub-national to Global), (b) Is a ChF assigned to an object in the biosphere: specific location, or a specific organism (man?), (c) Is the number of chemicals involved one, several, all?, (e) Are chemicals treated as individuals, or grouped, or aggregated by means of toxicity related summation (TCDD-TEQ, UseTox, else). (f) What position to indicate in the cause-effect chain: from occurrence in the technosphere, to the "n-th" order effect in the environment? (f) Are also metabolites included?, and (g) What are relevant impacts, i.e. human health, or ecosystem integrity (only), or also e.g. photo chemical oxidant formation, among others? Given the apparent versatility of the concept and its potential use in chemicals management, a substantial motive to collate the initiatives exists. A SETAC-Working group would be a functional way forward with the goal to e.g.: 1) frame the existing methodologies according to applications and 2) evaluate and fill gaps and weaknesses of proposed methodologies. Input from both the RA and LCA communities are necessary to reach sound and versatile methods which are useful for chemical risk reduction and management, and to underpin development towards the definition of a planetary boundary, or boundaries, for chemical pollution.

220

Initial risk assessment of mixtures of plant protection products

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In 2013, more than 100 active compounds of plant protection products (PPPs) are approved for use in Norway. Even though PPPs are risk assessed individually, effects on organisms in the aquatic environment might occur through combined toxicity as co-occurrence of PPPs in agricultural streams are more the rule than the exception. In this study a cumulative risk assessment based on available effect and exposure data was performed to assess the environmental risk of mixtures of PPPs to non-target organisms. Effect data for algae, crustaceans, fish and aquatic plants were collected from various databases and used for calculating predicted no effect concentrations (PNEC). Measured environmental concentrations (MEC) of PPPs at six different sampling sites in Norway were obtained through the Norwegian Agricultural Environmental Monitoring Program, JOVA. The composition and concentrations of PPPs varied between the different sampling sites and different sampling time-points. A risk quotient based on the sum of MEC/PNEC ratios of the detected PPPs in each sample ($RQ_{MEC/PNEC}$) was calculated. In addition, a taxa-specific risk was calculated by summing the toxic units (TU) to obtain the risk quotient RQ_{STU} after application of an assessment factor of 100. Risk was identified when RQ > 1. Of the total 56 samples, eight had a calculated $RQ_{MEC/PNEC}$>1; two samples from Hotranelva, four samples from Mørdrebekken, one sample from Skuterudbekken and one sample from Vasshaglona. The cumulative risk was lowest at Timebekken ($RQ_{MEC/PNEC}$ of 0.121) and highest at Skuterudbekken ($RQ_{MEC/PNEC}$ of 32.5). The identified risk scenarios based on $RQ_{MEC/PNEC}$ were confirmed by RQ_{STU} values above 1 for aquatic plants based on the samples from Hotranelva, Mørdre and Vasshaglona, and for algae in samples from Vasshaglona and Skuterudbekken. The risk at each site appeared to be driven by only a few of the detected compounds. As risk assessment of combined toxicity of complex mixtures is still in early phases of development, a need for in-depth experimental and theoretical effort to improve both the data support and verify the predictive

modeling approaches were identified. However, the results obtained in this study and previous studies utilizing similar approaches suggest that summation of MEC(PEC)/PNEC ratios appear to be an acceptable approach for initial risk assessment of mixtures of PPPs.

221

Mixtures in the environment – development of assessment strategies for the regulation of chemicals under REACH

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This poster / presentation describes and discusses approaches for the environmental risk assessment (aquatic compartment) of mixtures under REACH. Different types of mixtures are defined. The focus lies on technical mixtures and discharge mixtures. Cumulative and aggregated exposures are considered. A tiered component based approach for the risk assessment of technical mixtures is proposed. It links the state of the art in mixture risk assessment methodology with data requirements and the assessment philosophy according to REACH. Therefore the use of REACH generated data, necessary amendments, and feasibility constraints under REACH in order to perform such a mixture risk assessment are analysed. As possible supplemental elements, mixture assessment factors and whole mixture testing are considered. Current limitations for risk assessment of technical mixtures under REACH are identified and acknowledged. Those are, inter alia, the generic and very crude substance exposure levels (PECs) generated by REACH risk assessment tools, the disparity in the availability of suitable data across the supply chain limiting the possibilities of different actors to assess mixture risks and the missing link between the responsibilities of the single REACH actor (producing or using technical and discharge mixtures and the components (quantitatively and qualitatively) of the actual local coincidental mixture in the receiving water volume which, however, determine the real environmental risk. Priority setting is essential for the risk assessment of mixtures. For this purpose, "Mixture Assessment Triggering Substances (MATS)" are proposed. MATS are selected based on single substances' risk related data indicating a relevant contribution to mixture effects according to concentration additivity assumptions, if they are present in a specific technical mixture. Further approaches for the identification of priority mixtures refer to critical components in mixtures, critical composition and critical uses of mixtures. End-users of technical mixtures can focus on aggregated exposures due to the parallel use of the same substance in more than one technical mixture. Options to assess technical mixtures under REACH have been developed for industry and for authorities. The feasibility of these options is analysed. Possible next steps for validating and refining the proposed mixture risk assessment strategy and for implementation are described.

222

SINGLE AND MIXTURE TOXICITY OF PHARMACEUTICALS ON ATYAEPHYRA DESMARESTII IN GLOBAL CHANGE SCENARIOS

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Lethal, sublethal and mixture toxicity tests of three pharmaceutical compounds Diclofenac (DF), Ibuprofen (IB) and Carbamazepine (CBZ) were carried out with the freshwater shrimp *Atyaephyra desmarestii* at two different temperatures (20º and 25ºC). Obtained lethal concentrations (LC_{50}) for each individual compound after 96 hours of exposure were 6.3 $mg{\cdot}L^{-1}$, 13.3 $mg{\cdot}L^{-1}$ and 94.3 $mg{\cdot}L^{-1}$ in organisms exposed at 20ºC and 6.4 $mg{\cdot}L^{-1}$, 10.1 $mg{\cdot}L^{-1}$ and 66.4 $mg{\cdot}L^{-1}$ at 25ºC for DF, IB and CBZ respectively. Based on obtained mortality data, Predictive No Effect Concentration (PNEC) was calculated for each of the compounds and compared with Predicted Environmental Concentrations (PEC) reported in surface waters. Environmental risk of each compound was estimated as the ratio between PEC/PNEC and revealed that IB could represent a medium risk in freshwater environments. Additionally, binary and ternary mixture toxicity assays of the selected compounds were carried out. The obtained data was applied to two predictive toxicity models: Concentration Addition (CA) and Independent Action (IA). Finally, risk assessment was carried by estimating risk quotients (RQ). In this study, the mortality produced by binary mixtures at 20°C is lower than that produced at 25°C and the predictive model that fits best is CA presumably because two of the compounds (DF and IB) present a similar mode of action. The selected sublethal endpoints: food ingestion, osmoregulatory capacity and respiration rates were monitored at environmental relevant concentrations, ranging between 10-70$\mu g{\cdot}L^{-1}$ for the three selected compounds. No significant differences in both ingestion rate and haemolymph osmolality were found between controls and treatment (Dunnett´s test p>0.05) neither at 20º nor 25ºC. A decrease in the degree of oxygen independence was observed in shrimps exposed to DF at 20ºC under anoxia conditions (1 mg $O_2{\cdot}L^{-1}$). For respiration rates there was a decrease in the oxygen consumption in organisms exposed to CBZ at 25ºC even in well oxygenated water (5 mg $O_2{\cdot}L^{-1}$). Ecotoxicological action models can be considered useful tools to assess the expected effects of complex mixtures of pollutants in the environment. The results obtained provide relevant information on the combined effect of contamination and temperature variation in aquatic organisms in scenarios subject to global warming.

223

Influence of environmental factors on the joint toxicity of chlorpyrifos and mancozeb to the terrestrial isopod Porcellionides pruinosus

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The increasing evidences that environmental factors can act along with pesticides, influencing their individual effects, have been attracting a growing attention between ecotoxicologists. Albeit the considerable amount of work published on this area in the last few years, the influence of environmental conditions on the behaviour and toxicity of pesticide mixtures remains still poorly understood. Aiming to contribute for this discussion, in this work, we evaluated the joint effects of the insecticide chlorpyrifos and the fungicide mancozeb to the terrestrial isopod *Porcellionides pruinosus*, under several temperature and soil moisture conditions. Both these pesticides are extensively used in several crops and their application is frequently simultaneous. A full factorial design, with three treatments of each pesticide, was repeated for each abiotic condition. The experiments lasted for 14 days, and the endpoints assessed included survival, consumption ratio and biomass gain/loss. The reference model of independent action was used in order to evaluate if there were any deviations to the additivity pattern, that could possibly be related to the different environmental conditions assessed. Despite the absence of interactions registered between these pesticides on survival, in some situations, this parameter did, however, differed with the environmental conditions. For instance, different survivals were found when exposing isopods to mixtures containing the same pesticide concentrations at different temperatures. Moreover, the significant effects registered for the feeding parameters, suggest that long-term consequences on the populations dynamics can occur. Since this species' ecological role is mostly related with its feeding activity, this can also entail deleterious effects on the edaphic ecosystems they are in.

224

Integrated chemical and biological assessment of sub-lethal toxicity of binary mixtures of Zn, HTO and DOC to Mytilus galoprovincialis

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Concerns over the presence of radioactive contaminants in the marine environment are growing in the light of recent nuclear accidents and the effects rising sea levels may have on the mobilisation of radionuclides from contaminated soils in coastal locations. Therefore, it is a high priority for scientific and regulatory bodies to gain a deeper understanding of the potential impacts of ionising radiation on aquatic biota and to derive appropriate standards to protect the environment from radionuclides, which are often co-disposed with (more abundant) stable metals. This is particularly the case for chronic exposure of marine invertebrates to low levels of metals or radionuclides. This study contributes data to hazard and risk assessment by elucidating the mechanistic processes and quenching effects that arise when marine species are subjected to binary combinations of metals or dissolved organic carbon (DOC) with radio-isotopes. The response of a model species (*Mytilus galoprovincialis*) exposed to a fixed concentration of a radionuclide (5 MBq L^{-1} tritium, HTO), with varying concentrations of a trace metal (25, 125, and 250 $\mu g\ L^{-1}$ Zn) or a source of DOC (0.1, 1, and 10 mg L^{-1} humic acid), was investigated. The study reports the biological effects at gene and DNA level and their potential link to physiological effects (e.g. feeding rate/ clearance rate). The speciation of zinc, a ubiquitous trace element with numerous estuarine sources and a UK Specific Pollutant under the EU Water Framework Directive, and its influence on the biological response of the test species will be discussed. \n

Chemical pollution in sustainable management of aquatic ecosystems – challenges and approaches from a

Swiss perspective (I)

225

Future challenges for the Swiss water sector: outlook based on the National Research Program Sustainable Water Management

C. Stamm, Eawag / Uchem; E. Rahn, R. Siber, K. Lanz, Eawag

Water is a vital resource for a large variety of sectors. Different societal needs and demands such as drinking water production, flood control, energy production and a good ecological status of water bodies may be in conflict with each other, but may also profit from potential synergies. Future oriented water management needs to be based on a comprehensive understanding of the societal sectors affecting water resources and how they interact under present and future conditions. As a contribution to the Swiss National Research Programme 61 on Sustainable Water Management, we investigated trade-offs and synergies among the various water-related societal objectives. The analysis used the present situation as a starting point and expanded to projected future changes due to climate and societal changes. The analysis focused on four dimensions: water quantity, water quality, hydromorphology and finally water conflicts as a consequence of land-use. This presentation summarizes the main findings and aims to put the issue of water quality into a broader context. On the one hand, existing conflicts such as the demand for increased hydropower generation and its impact on stream ecology will continue to be relevant topics in the future. In addition, new issues are developing, for instance the steady increase of heating and cooling uses of surface and groundwater, or conflicts over land-use between urban development and drinking water protection areas. Regarding water quality, Swiss politics have recently decided to invest substantially in the removal of micropollutants from wastewater, intending to markedly reduce their input into the aquatic environment over the coming years. However, this measure will only affect about 10% of the stream network. Hence, to further improve aquatic ecology, diffuse pollution with pesticides and heavy metals will have to be tackled more stringently in the future. Industry is another as yet insufficiently understood source of potential pollution in Switzerland. These qualitative aspects of Swiss water bodies require inspection from a broader perspective. This also applies to on-going river restoration programmes (4.000 km over the next 80 years) which may yield unsatisfactory outcomes as they consider only part of the ecological stressors. Quantitative aspects which long seemed secondary due to the abundance of Swiss water resources were identified to pose an additional challenge, mainly for agricultural development under warmer and potentially drier summer conditions.

226

The chemical status of Swiss surface waters and the role of diffuse pollution with focus on pesticides

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Diffuse pollution in surface waters includes inputs from many various sources and substances, like i.e. plant protection products (PPP) or animal drugs from crop land, biocides from facades in housing areas or heavy metals in road runoff. One of the main challenges to monitor inputs of micropollutants from diffuse sources is the mostly rain-driven dynamic release to surface waters (i.e. runoff). Pesticides are clearly one of the most important substance groups regarding diffuse pollution and one of the most intensively monitored substances in Switzerland. In Switzerland, surface water monitoring, including the assessment of pesticide exposure, is mainly the task of the 26 cantonal authorities. Between 2005 and 2012 more than 345’000 concentration data of 203 pesticides, either registered only as PPP (n= 149) or biocides (n=18) or for both uses (n= 36), were measured at 565 sampling sites in Swiss streams. This study presents the main results of the first nation-wide compilation of pesticide data from cantonal and federal monitoring data. 98 of the 162 detected pesticides were measured above the numerical requirement of the Swiss law of 0.1 µg/l. These exceedances were observed at over 70% of all sites. 18 pesticides (only 1 biocide) were even measured at concentrations above 10 µg/l. Of the pesticides with the highest maximum concentrations, only one is registered as biocide, the rest are PPP or registered for both uses. The highest concentrations were found in small streams (Strahler order 1 and 2). Although the stream network in Switzerland consists of 75% of small streams most of the sampling sites are located at medium-sized or large streams (Strahler order 3 to 9), thus small streams being clearly underrepresented in the data set. Most samples were taken as monthly grab samples or composite samples; high resolution monitoring data were scarce and thus peak concentrations most probably rarely captured. When comparing highly resolved monitoring data of small catchments with the whole data set, maximum concentrations can be up to one order of magnitude higher. Further, the combination with land use data indicates that this elevated pesticide exposure can be widely expected in the small streams of the Swiss Plateau. It can be concluded that due to the site selection and the applied sampling strategies data collected in standard monitoring programs represent only the lower limit of the real pesticide exposure in small streams.

227

Effects of micropollutants on fishes and macroinvertebrates

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228

EcoImpact – assessing the ecological impact of micropollutants in Swiss streams

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There is mounting concern about the impacts of human populations on biodiversity and ecosystem services at a global scale, although many of the effects contributing to these changes are exerted at spatially discrete, local scales[1,2]. These concerns, in part, have generated questions of scaling impacts across differing levels of biological organisation (e.g., genes to ecosystems) and their inherent spatio-temporal arrangement, leading to the development of macroecology and ‘macrosystems’ ecology as emerging sub-disciplines within the biological sciences[3,4]. Stream ecosystems are model examples of spatially discrete habitats with hierarchical linkages to larger receiving environments, and where adverse changes are often manifested through increased environmental degradation[2,5] with complex outcomes mediated through interactions of multiple stressors[6,7]. In particular, micropollutants (e.g., low concentrations of pharmaceuticals and pesticides) from municipal wastewater and intensive agriculture may be a serious threat to the biodiversity and ecology of streams and their receiving environments[8]. Efforts to improve point-source pollution through advances in water-treatment technologies have led to the beneficial upgrading of municipal wastewater treatment plants (WWTPs) in developed countries including Switzerland. However, although nutrient loads have been greatly reduced, the biological consequences that wastewater discharges and associated micropollutants, which can bypass conventional treatment methods, have in real ecosystems remains poorly understood. In particular, there is a deficit of knowledge in understanding the impacts that mixtures of low-concentration toxic compounds have on receiving environments, and if changes are mediated through indirect effects (e.g., species interactions), in addition to the direct effects of acute and chronic toxicity. To address these knowledge gaps, we are assessing changes to stream ecosystems through observational and experimental approaches using a range of structural and functional indicators. EcoImpact is an ambitious Eawag project testing the effects of micropollutants entering stream ecosystems via wastewater treatment plant (WWTP) discharges in Switzerland. There are two main components to the study; an observational approach involving field surveys and the other using flow-through stream flumes for an experimental approach. For the observational component of the study, 24 sites across the Swiss Plateau are to be surveyed over two years. Currently, data from 12 streams has been collected above and below WWTP discharges, with basic water chemistry, macroinvertebrate communities, and ecosystem functions, including decomposition and stream metabolism recorded. Almost 400 micropollutants are currently being screened from water samples collected at study sites. This approach is useful for assessing existing influences of WWTP discharges on stream ecosystems, but also provides baseline data to help evaluate the effectiveness of system upgrades in the future. However, basic water chemistry data collected monthly provides confirmation of the altered stream environment below wastewater (WW) discharges, and the majority of variables measured, including nutrients, show elevated concentrations downstream. Analysis of stream invertebrate data using permutational multivariate analysis of variance (PERMANOVA) shows that community composition changes below WW discharges. This change reflects in part increased numbers of oligochaete worms and decreased abundances of baetid mayflies, leading to a large decrease in community evenness. Using the BETADISPER command in R demonstrates that this reduction in beta-diversity potentially also leads to more nested community structure exposed to WW, as evidenced by the lower dispersion of downstream sites in community similarity plots. Similarly, SPEAR (Species at Risk) indices and reduced EPT species diversity confirm these patterns by showing that abundances of pollution-sensitive taxa decrease below the WW discharge. Partial redundancy analysis (*p*RDA) of relative abundance data indicates that water chemistry variables appear to explain the most variation in community abundances after partitioning out the influences of habitat and spatial location. Interestingly, these structural changes to stream invertebrate communities may be associated with impaired and/or altered ecosystem functioning. Preliminary analysis of litter breakdown assay data shows that breakdown rates of leaves were lower in coarse-mesh bags below the WW discharge, thus indicating decreased function by ‘shredding’ invertebrates. In contrast, fine-mesh litter bags (excluding invertebrates) showed a small positive increase in litter decomposition, suggesting a positive influence of WW on microbial processes. Using the cotton-strip assay, another functional indicator of decomposition, confirmed this pattern by showing increased microbial activity and cellulose fibre degradation below the WW discharge. These changes were apparent in measures of ecosystem metabolism using single-station diurnal oxygen logging,

which showed increased community respiration and a shift to a more heterotrophic state. In summary, our findings show that discharges from WWTPs significantly alter stream-water chemistry and influence invertebrate community composition and ecosystem functioning. Less clear, however, are the links between structural and functional changes, and the underlying processes driving apparent effects (e.g., the contribution of micropollutants remains unresolved). However, the observational data we have collected from the field survey helps to better formulate hypotheses that will be tested using the flow-through stream flumes. Thus, in the future, the functional consequences of structural changes and underlying mechanisms will be investigated more rigorously through experimentation.

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Fish model species in environmental toxicology (II)

229

Benefits and limitations of using fish embryos in applied ecotoxicology

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Over the last six years, we have been exploring the scope of the fish embryo toxicity assay (FET) for testing different chemicals of a wide mode of action range in terms of sensitivity, meaningfulness and practicality. Many lessons have been learnt from this endeavour, but the overall résumé shows that this test is a resource- and powerful tool to study toxicity at different biological levels. The aim of this presentation is to summarise some interesting results and key outcomes from our investigations into the practical application of the FET. Examples of studies of different exposure scenarios and with different compound groups will be shown to demonstrate how the conventional FET can be enhanced and refined by combination with additional cellular and molecular methods. Correspondingly, the test has certainly some limitations, which have to be considered and recognised, but these are by far outweighed by the benefits. Fish embryo toxicity tests were conducted, using different exposure times from 48 to 120 hours post fertilisation. Most FETs were performed using wild-type zebrafish; however for investigations on mode of action specific effects, like vasotoxic or neurotoxic effects, embryos of transgenic zebrafish lines were used. Post exposure analysis methods ranged from fluorescent immunohistochemistry to acridine orange vital staining for apoptosis detection to RNA extraction with subsequent gene expression analysis using real-time PCR or microarrays. Exemplary results of studies investigating endocrine disrupters, nanoparticles and other specific MOA chemicals demonstrated that the FET is more than just an alternative for the acute fish toxicity test. Transcriptome data of 48h embryos e.g., showed that estrogenic chemicals can be discerned from anti.androgenics based on the expression profile and the regulation of specific signalling pathways. Also,chemicals affecting the vascular or the neuronal development of the embryos could be identified more sensitively with transgenic zebrafish than using the conventional FET with wild-type embryos. Overall, the FET offers huge potential as an animal alternative toxicity test when its limitations are taken into consideration.

230

Impact of an agricultural contaminant on reproductive behaviour and morphology in a freshwater fish

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One class of chemical pollutants, known as endocrine disrupting chemicals (EDCs), have the capacity to disrupt the natural endocrinology of organisms. While the morphological and physiological consequences of exposure to EDCs are well known, their effects on behaviour have received less attention. Further, of the behavioural studies to date, the vast majority have focussed on estrogenic endocrine disruptors, while the behavioural impacts of androgenic EDCs are less well understood. One androgenic EDC of particular concern is 17β-trenbolone. This chemical is a metabolite of the hormonal growth promotant trenbolone acetate, which is used globally in beef cattle production. Prior research has repeatedly identified 17β-trenbolone in the environment, and has established its ability to disrupt development and reproduction. The behavioural consequences of exposure to 17β-trenbolone are, however, poorly understood. The aim of this study was to investigate the impacts of short-term (21-day) exposure to an environmentally relevant concentration of 17β-trenbolone (22 ng/L) on male and female reproductive behaviour and morphology in the guppy, *Poecilia reticulata*. Male guppies use two alternate mating strategies, either courting females to solicit copulations or achieving forced copulations via sneaking behaviour. The relative usage of these tactics varies with environmental conditions, providing a useful framework to analyse the effects of EDCs. To investigate the impact of 17β-trenbolone on guppy reproductive behaviour, four treatments were employed: (1) unexposed male with unexposed female, (2) unexposed male with exposed female, (3) exposed male with unexposed female, and (4) exposed male with exposed female. Our study revealed that exposure to 17β-trenbolone, for males, was associated with changes in the total duration and number of courting events, and the number of sneaking attempts, performed across treatments. However, no effect of 17β-trenbolone was detected in females, indicating sex-specific vulnerability at this dosage. This study is the first to show altered reproductive behaviour in male animals in response to an environmentally relevant exposure to 17β-trenbolone. Given potency and ubiquity of 17β-trenbolone, continued multidisciplinary scrutiny of this EDC is necessary to reveal the consequences of its presence in the environment. Keywords: endocrine disrupting chemicals, sexual selection, behaviour, fish

231

Use of an estuarine model teleost to evaluate pollutant impacts at multiple biological scales

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Estuarine teleosts are rarely used in the study of fish ecotoxicology, and hence knowledge is sparse for many important species. As such, we have developed the euryhaline species *Menidia beryllina* as an model for the study of endocrine disruption at the gene, protein, reproductive, and population levels. We chose to evaluate bifenthrin, a pyrethroid frequently utilized for urban and agricultural applications. Bifenthrin acts as both an estrogen receptor agonist and antagonist at the pptr concentrations commonly present in aquatic ecosystems. Recently, additional concerns have arisen regarding the endocrine activity of bifenthrin metabolites. As such, our experiments seek to expand upon what is already known regarding the mechanisms by which bifenthrin interferes with endocrine signaling in fish and to link changes in gene and protein expression with higher biological scales. We conducted exposures to three pptr concentrations of bifenthrin and evaluated the expression of a suite of endocrine genes. We found that these genes were down-regulated in response to bifenthrin exposure, indicating either antagonism or negative feedback mechanisms. We also conducted 7d exposures to pptr concentrations of a bifenthrin metabolite and to a mixture of bifenthrin and piperonyl butoxide, which inhibits P450 enzyme activity, to determine the role of metabolism. Results of these experiments indicate that bifenthrin increases the expression of the estrogen-dependent protein choriogenin, and that bifenthrin metabolite(s) appear to have greater estrogen agonist activity than the parent compound. Results from a newly-developed *Menidia beryllina* microarray and RNA seq further clarify the mechanisms underlying bifenthrin’s modulation of the endocrine system. These results, along with a current evaluation of reproductive behavior, will be considered in the context of reproductive output via data collected from spawning trials, which found reduced egg output after 7d exposure to 0.5 ng/L bifenthrin. Furthermore, a population model parameterized with these results indicates the potential for population decline at these exposure levels. This model can be used both in the lab and in the field to examine the effects of emerging contaminants and could be used as a surrogate for other estuarine fishes. \n

232

Sentinel medaka detect multiple mechanisms of steroid signalling disruption

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Aromatase is the key enzyme in regulating the balance between androgen and

oestrogen signalling. Development of *in vitro* tests to identify chemicals altering the expression or activity of aromatase has shed light on their widespread use, particularly in agriculture. Another key enzyme involved in steroid signalling is 5α-reductase which is responsible for the conversion of testosterone into the more powerful androgen dihydrotestosterone (DHT). To carry out large scale screening programs to identify endocrine disrupting chemicals (EDCs) altering oestrogen signalling, rapid and robust screening tools are required capable of identifying EDCs not only acting at the receptor level but also interfering with steroid metabolism. The use of pre-feeding aquatic larvae presents the advantage of being able to perform experiments in multi-well plates. We present a novel and robust exposure protocol for use with medaka fry harbouring the ChoriogeninH-GFP (ChgH-GFP) transgene. Exposure studies were carried out on 20 ChgH-GFP medaka fry per group exposed in 7ml of medaka medium. Exposures began at day post hatch zero for a duration of 48 h. Solutions were renewed every 24 h and fluorescence was quantified every 24 h. All test substances were tested in the presence and absence of testosterone. Colour images of the ventral region of the abdomen of each fry were captured at 8x magnification using GFP longpass filter sets. Background fluorescence of the fry resulting from endogenous pigments was removed by subtracting the red layer from the green layer and applying a 10-255 threshold. GFP signal was generated in response to exposure of fry to the aromatisable androgen testosterone. The lack of signal observed when fry were cotreated with testosterone and the pharmacological aromatase inhibitor anastrozole demonstrated that conversion of tesosterone to oestradiol via aromatase is necessary for a fluorescent response. By exposing ChgH-GFP fry to a variety of chemicals, we determined that EDCs acting via at least seven different mechanisms of action can be identified using ChgH-GFP fry exposed in the presence or absence of testosterone. The protocol described here provides a reliable method to screen potential EDCs acting on the oestrogen axis via a variety of mechanisms. In addition this protocol provides information on steroid signalling from an entire intact organism whilst complying with the three R's principle of animal replacement.

233

Assessment of the estrogenic potency of chemicals, alone or in combination, and complex environmental mixtures using a novel transgenic cy19a1b-GFP in vivo zebrafish assay (EASZY assay)

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One major challenge to ecotoxicology is to develop species-specific biological tools that allow rapid and cost-efficient assessment of Endocrine Disrupting Chemicals (EDCs) for environmental hazard and risk assessment but also for monitoring the contamination of aquatic systems by EDCs. In this context, the use of zebrafish (*Danio rerio*) embryo assay allowing the detection and quantification of EDCs based on their mode of action appear as usefulness and relevant to address these issues. We recently developed a mechanism-based *in vivo* zebrafish assay called EASZY that allows the detection of Endocrine Active Substance, acting through zebrafish estrogen receptors, using transgenic cyp19a1b-GFP Zebrafish embrYos (EASZY). The Cyp19a1b gene is a strictly ER-regulated gene that codes for the brain aromatase which is mainly expressed in radial glial cells of the brain of fish. In the present work, the relevance of EASZY assay for assessing the estrogenic potency of substance was evaluated by testing more than 50 different compounds belonging to various chemical classes. We found that in EASZY, GFP is induced in a ER-specific manner by (i) compounds that bind directly to estrogen receptors as agonists (ii) compounds that require metabolic activation into estrogenic metabolites (iii) aromatizable androgens as well as some non aromatizable androgens. By studying combined effects of estrogenic chemicals, we showed that mixtures of estrogenic compounds generally acted in an additive manner on *th*e expression of GFP, their combined effects being predicted by the CA model although antagonism was observed in a specific case. Finally, Polar Organic Chemical Integrative Sampler (POCIS)-based bio-monitoring using EASZY allowed screening for estrogenic activity of various surface waters from French rivers resulting in the detection of estrogenic activities at some sites. Overall, our studies show that EASZY assay clearly emerges as a simple, fast and reliable *in vivo* assay for screening the capacity of chemical, alone or in combination, as well as complex environmental mixtures. The sensitivity of EASZY assay to activate ER-signalling by chemicals and complex mixtures at very early critical developmental stages makes it an outstanding screening in vivo tool for hazard assessment of chemicals and EDCs detection in aquatic environment.

Acknowledgement – Study support by INERIS (P190 Ecotoxicologie) and ONEMA

234

Application of Molecular Methods for Understanding the Effects of Endocrine Disrupting Chemicals in Fish Models

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A wide range of chemicals are now known to mimic hormones structurally or interfere with their biosynthesis and/or degradation. Exposure to these so called endocrine disrupting chemicals (EDCs) can cause disruptions in development, alter sexual differentiation and function, and impact adversely on reproduction. In this presentation, we illustrate the application of a suite of oestrogen receptor (ER) reporter gene assays, transgenic fish and DNA microsatellites to advance understanding on the mechanisms of oestrogen action in fish and the functional consequences for both individuals and populations. Fish ERs in reporter assays showed similar responses to the natural ligand oestradiol-17b (E2, and also for the oestrogen receptor subtypes) but very different responses to oestrogen mimics, with implications for risk assessment. We show that the ligand binding domain of the ER confers differences in responsiveness to oestrogen mimics between different fish species. Applying an oestrogen responsive element transgenic (ERE-TG) biosensor zebrafish (ERE-TG fish) we show that a variety of tissues respond to oestrogens, including the liver, heart, muscle and forebrain illustrating the wide potential health effects associated with oestrogen exposure. Exposure studies with BPA reveals valves in the heart as a target site and the breakdown product of BPA, MBP,is around 1000X more potent that the parent compound. Male roach (*Rutilus rutilus* L) feminised by exposure to oestrogenic effluents can be compromised in their reproductive fitness however using DNA microsatellites, we did not find that this impacted on the effective population sizes (N_e) of wild roach (*Rutilus rutilus* L) living in UK rivers, and it appears that populations in oestrogen contaminated environments were self sustaining.

Wildlife ecotoxicology: from acute toxicity to low level, chronic exposure related effects (II)

235

Energy normalized diet concentrations as measure for secondary poisoning of birds and mammals

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The risk assessment of secondary poisoning of birds and mammals is usually performed by either the dietary approach or the dose based approach. Both methods however, have their disadvantages. The dietary approach does not take into account the differences in energy content between food items, leading to sometimes substantial differences in food intake. This concerns both the food provided in the laboratory toxicity studies and the food items from the field (e.g. fish, bivalves or earthworms). The dose based approach actually only considers the intake rate of the chemical. Because the dose is the highest for small mammalian and avian species, these species are selected as indicator species for this approach. The species with the highest intake rate will have the fastest buildup of the chemical concentration in the organism. This is relevant for acute poisoning, as often addressed in risk assessment of pesticides. However, also the clearance rate is equally dependent on the body weight, and therefore, upon long-term exposure these effects will cancel each other. The dose might therefore be a less suitable metric for long-term exposure. A method is presented here, which uses dietary concentrations normalized to the energy content of the diet. Diet concentration can be directly normalized to the energy content, or the normalized concentration can be calculated from the dose and the ratio of daily energy expenditure and body weight. Daily energy expenditure can be accurately estimated from body weight. Chronic toxicity of hexachlorobenzene for mink was taken as an example. Both methods to calculate the diet concentration normalized to energy content gave very similar results. This value can then be recalculated to different food items relevant for the food chain considered (freshwater, marine, terrestrial) by means of the energy content of these food items. Together with the bioaccumulation parameters it can be decided which food item is critical for the food web. The method differentiates better between different food items. Further, it is flexible, because it can be applied to less common food items as well.

236

Can stable isotopes explain contaminant load in birds of prey?

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Measurement of polychlorinated biphenyls (PCBs) and mercury (Hg) in the eggs of predatory birds has been undertaken as part of the long-term monitoring work of the UK Predatory Bird Monitoring Scheme (PBMS—http://pbms.ceh.ac.uk). We have measured PCB and Hg concentrations in the eggs of a marine sentinel species, the Northern gannet (*Morus bassanus*) from two Scottish colonies (Ailsa Craig in the Eastern Atlantic and Bass Rock in the North Sea). We have also monitored these contaminants in the eggs of golden eagles (*Haliaeetus albicilla*), a species of high conservation concern in Britain. Our analyses revealed higher levels of

contaminants in the eggs of Ailsa Craig than Bass Rock gannets and in the eggs of coastal nesting compared with inland nesting golden eagles. The intra-specific differences in gannet eggs have been assumed to reflect colony-differences in diet and/or trophic level position, although alternatively there could be differences in contaminant loadings between eastern Atlantic and North Sea fish. The higher contaminant loadings in coastal-nesting than inland nesting golden eagles have been attributed to coastal nesting birds preying, at least partly, on seabirds and being exposed to marine contaminant pathways. However, there is no real information to support the assumptions about the role of diet influencing egg contaminant loads for either species. We measured stable isotopes (^{15}N, ^{13}C and ^{34}S) in eggs as proxy indicators for diet to test if intra-specific differences in contaminants could be explained by differences in diet. We used $\delta^{15}N$ as a marker of trophic position, $\delta^{13}C$ as a marker of the carbon source in the diet and $\delta^{34}S$ a marine marker. The gannet eggs from Ailsa Craig were significantly more enriched in δC^{13} and δN^{15} than those from Bass Rock, suggesting increased consumption by Ailsa Craig gannets of higher trophic level prey and/or an increased proportion of demersal fish discards in the diet. This may account to some extent for the higher levels of ΣPCB concentrations and Hg concentrations in eggs from Ailsa Craig.

237

Effects of As, Cd and Pb on blackbirds: a multi-marker study

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The present study aimed to get insights into the pathways linking effects at individual and population level and how gender-specific responses may affect such links. We used a multi-marker approach on the European blackbird *Turdus merula* in order to investigate population structure parameters, potential metal toxicity with health biomarkers (haematology, blood biochemistry/enzymatic activities, oxidative stress), potential depletion of quantity/quality of food with nutritional status biomarkers (body condition index, blood biochemistry), and relate these different parameters with exposure to As, Cd and Pb (blood and feather concentrations). Eighty-three blackbirds (55 males, 28 females) were captured during the breeding season in five sites along a pollution gradient in Antwerp (Belgium). The levels of As, Cd and Pb in tissues increased along the pollution gradient and did not vary with gender. Levels in blood and feathers were highly correlated, strongly suggesting that birds were chronically exposed. The sex-ratio did not vary with pollution. The age structure, for males only, differed between sites with an increase of yearling proportion in the most polluted sites, which may be interpreted as a result of higher mortality or emigration in these polluted sites. The proportion of female carrying a visible egg decreased with pollution level although not significantly. No relationships were observed between exposure and markers related to nutritional status. In males, the results suggested some health disorders (anemia, myopathy, kidney dysfunctioning and metabolic homeostasis disorders). No oxidative stress was detected but a modification of oxidative status, with an increase of some circulating antioxidants (lutein, tocopherol) with metals. In females, only few correlations were found, most of them being significant only for females carrying an egg. Like in males, no oxidative damages were detected but an increase of lutein with metal levels. A decrease of GSH with metals, not observed for males, was also found. Altogether, the results suggested a higher sensitivity of males to toxic effects, but may also mirror trade-offs between reproduction and individual survival in the most polluted sites for females. The modification of circulating lutein and tocopherol may be related to the effects of metal(loid)s on oxidative status, but may also be linked to changes in hormone levels, notably stress hormones.

238

Are immunotoxic effects of lead shot ingestion linked to oxidative stress?

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The ingestion of lead (Pb) shot used for hunting constitutes the main cause of Pb poisoning in avifauna, but very little is known about the sublethal effects on birds and their consequences at a population level. We studied the relationship between Pb shot ingestion, oxidative stress biomarkers and the immune function in red-legged partridge (*Alectoris rufa*). Partridges (n=60) were housed in pairs and assigned to three experimental groups: Control (no shot), low dose and high dose. Pb concentration and δ-aminolevulinic acid dehydratase (δ-ALAD) activity, were determined in blood. Malondialdehyde concentration (MDA), total glutathion levels (GSH) and superoxide dismutase activity (SOD) were quantified in red blood cells homogenates. Vitamins (retinol, α-tocopherol) and carotenoid (lutein) levels were analyzed in plasma. Carotenoid-based coloration was studied by digital photographs and beak and eye ring spectrophotometry. Cell-mediated immune and humoral responses were studied using the phytohemagglutinin (PHA)-skin test and the haemagglutination test after antigen injection, respectively. Acute-phase and antibacterial proteins were measured in plasma. Constitutive immune response was tested determining bactericide activity of fresh whole blood. A negative relationship between Pb concentration and δ-ALAD was found. Pb exposure increased levels of GSH and the primary inflammatory response to PHA. In females, δ-ALAD activity was positively correlated with intensitiy of carotenoid-based red coloration and with relative amount of the eye ring area pigmented by carotenoids. GSH concentration was positively associated with blood Pb concentration. Cellular immune response increased with Pb exposure, while humoral immune response decreased. Exposure to Pb in males decreased retinol and lutein levels in plasma. Antioxidant mechanisms appear to be affected by Pb exposure in a different way in females and males. Females may show a hormetic response with the increase of GSH and enhanced cellular immune respose, but at the cost of a loss on carotenoid ornamentation and a reduced humoral immune response. Males exposed to Pb maintained the pigmentation of ornaments and the competence of both immune functions, but showed a more marked decrease of plasma antioxidants. This may reflect a handicap of the carotenoid-antioxidant allocation trade-off in males between the expression of sexual signals and the immunitary response or other oxidative stress-sensitive functions.

239

In ovo exposure to BDE-99 affects the song control system of a model songbird species, the zebra finch

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2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) is a brominated flame retardant congener that has pervaded global food chains, being reported in avian egg and tissue samples throughout the world. There is evidence that BDE-99 has endocrine disrupting effects, and exposure to BDE-99 in the early life stages could have significant impacts on development. One component of the nervous that develops during the embryonic and early post-hatch period is the song-control system, which is involved in song learning and production. There are concentrations of androgen and thyroid hormone receptors in the song-control nuclei, and there is the potential that the development of the song control system could be affected by exposure to xenobiotics such as PBDEs. Singing is an important aspect of reproduction in birds, serving to define territories and attract females. Developmental conditions that result in smaller song-control nuclei and reduced song quality in males could ultimately disrupt pair formation and lower reproductive success. In the present study, the effects of *in ovo* exposure to environmentally relevant levels of BDE-99 were assessed in a model songbird species, the zebra finch (*Taeniopygia guttata*). Embryos were exposed via egg injection to a vehicle control (DMSO), 10, 100 or 1000 ng BDE-99/g egg on the day the egg was laid. Chicks were raised to sexual maturity to investigate long-term effects of BDE-99 on the adult male brain. Three key song control nuclei (AreaX, HVC, RA) all showed non-significant decreases in volume as BDE-99 concentration increased. As these three nuclei all correlated with each other, we used a principle components analysis of these three varaibles to get a single measure of the song control system. The first principle component (PC1) explained 60% of the variation in nuclei volumes. We compared PC1 scores across doses and found a significant effect on the song control system, with BDE-99 exposed birds having significantly smaller PC1 scores compared to control birds. This study has shown that current environmental levels of BDE-99 are high enough to have significant effects on the song control system in birds, and may therefore be impacting the reproductive rates of free-living birds. We have previously shown that there is no effect of BDE-99 exposure during the nestling period on the song control system. Taken together, these results demonstrate that the timing of exposure can significantly alter the effects of contaminant exposure.

240

The effects on steroidogenesis and cellular structures of adult male Japanese quails (Coturnix coturnix japonica) testis following pre-pubertal exposure to di (n-butyl) phthalate (DBP)

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Phthalates esters (PE) are synthetic chemicals widely used as plasticizers and ubiquitous in the environment. Many laboratory studies have shown generally that phthalates, including the di (n-butyl) phthalate (DBP) are anti-androgenic causing adverse biological effects on male reproduction, growth and development in both human and wildlife. Phthalates are known to interfere with the transcription of several key genes involved in cholesterol transport and testosterone biosynthesis. The down-regulation of several genes involved in steroid biosynthetic pathways is thought to be central to phthalate-esters induced anti-androgenic disruption. In present study, we investigated the effects of 30d dietary (prepubertal) exposure to different doses (0 (control),1,10,50,200and 400 mg/kgbw)of DBP on testis (Leydig

cells) of adult male Japanese quails by quantifying the transcript expression of $P450_{scc}$, $P450c_{17}$, aromatase (cyp_{19}), androgen receptor, 3β-and 17β-HSD using qPCR. In addition, the plasma testosterone levels was analysed using radioimmunoassay; and testis was examined for evidence of gross- and histopathology. Our overall objective was to test the effects of DBP on cellular and molecular processes that underlie testicular steroidogenesis, using the male Japanese quail, as a sentinel avian model. We hypothesized that exposure of male quails to the environmental DBP at pre-pubertal period, will produce differential expression patterns of selected genes involved in testicular steroidogenesis, and may subsequently leads to alterations in testosterone levels and gonadal development. Our data showed that prepubertal exposure to DBP resulted in alterations in testicular architecture as evident by poorly developed or mis-shaped testis, and disturbed spermatogenesis due to degeneration and slight atrophy of seminiferous tubules especially in the DBP treated high dose groups. In addition, DBP disrupts several key enzymes involved in testicular steroidogenesis pathways in a dose-dependent manner. P450scc mRNA expression was up-regulated at low dose 10 mg/kg bw, and thereafter, a dose-dependent decrease (down-regulation) was observed between 50 and 400 mg/ kg bw. In conclusion, the present data is consistent with previous reports showing that DBP modulate Leydig cell steroidogenesis in several species, with a potential negative effect on reproduction in those avian species that are vulnerable to EDCs in the wild populations.

Innovations in environmental analytical chemistry: the quest for pollutants at trace levels (II)

241

The use of scripting filters for the identification of potential novel persistent organic pollutants following GC×GC-ToF MS analysis

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Comprehensive two dimensional gas chromatography coupled to mass spectrometry (GC×GC–MS) represents a valuable approach when dealing with the simultanous screening of different families of organic micropollutants in environmental samples. Depending on the complexity of the sample matrix, hundreds to thousands of peaks can be detected in a single analytical run. Interpretation of these large data sets can be a challenging task for the analyst, especially if some analytes are unknown compounds for which no MS data or information is available. The possibility of defining identification criteria could facilitate the interpretation of the data obtained. Recent studies have shown that threshold criteria can be used for the identification of potential bioaccumulative and persistent chemicals based on physicochemical properties. The majority of compounds classified as “potential POPs” are halogenated and therefore, pre-defined MS scripting filters for the determination of halogenated compounds during GC×GC–MS analysis can provide direct information regarding both conventional and unknown potential persistent organic pollutants (POPs) in the sample. With the focus on the goal of early identification of emerging halogenated substances, this study evaluates the use of a single script during GC×GC–MS analysis for the determination of potential novel POPs in sediments collected in Ontario (Canada).The script was designed to classify peaks based on the recognition of a common halogenated isotope pattern present in the mass spectrum of any compound that contains at least one chlorine or bromine. Once validated, the script was applied to sediments analysed by GCxGC–MS in order to detect prevalent halogenated compounds. A compound with a mass spectrum showing sequential loses of chlorines and bromines and with an odd molecular weight was detected in 27 out of 69 sediments. Ultra high resolution MS experiments indicated that the molecular mass of compound was 390.8162 da. The most logical molecular formula obtained from the elemental composition calculator was: $C_{12}H_5NCl_2Br_2$ (error: 0.438 ppm). A halogenated dibenzocarbazole was the most probable structure for the chemical. Extra experiments with different dibenzocarbazole isomer standards showed a good match with the 1,8-dibromo-3,6-dichloro-substituted isomer. This is the first time that a mixed halogenated carbazole has been identified in environmental samples.

242

Rapid post-screening of a sediment sample with complex matrices by GC × GC-HRTOFMS with R-based peak sentinel tool (T-SEN)

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Recently, comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC × GC-MS) draw attention as a powerful instrument for comprehensive analysis of organic pollutants. In this study, we developed an analytical method using GC × GC with data processing tool for rapid and accurate identification and quantification of compounds in environmental samples with complex matrices. Specifically, an automatic peak sentinel tool, (T-SEN), was developed with free programming software, R. This tool consists of a simple algorithm for peak finding based on a compound database and peak shape identification. It allows rapid screening of target compounds, even for large data sets from GC × GC coupled to high resolution time of flight mass spectrometry (HRTOFMS), typically up to 2 GB. The tool automatically assigns and integrates peak area of compounds that are listed in user databases. T-SEN for GC × GC-HRTOFMS works on a typical 64 bit workstation, and the reference calculation speed is 10–20 min for approximately 170 compounds for peak finding based on five compound-derived ion and integration from 1–2 GB of sample data. We demonstrated an ability to quantify 17 PCDD/F congeners and 24 PCB congeners in a crude lake sediment extract by both GC × GC coupled to quadrupole mass spectrometry (qMS) and GC × GC-HRTOFMS with T-SEN. Whereas GC × GC-qMS with T-SEN showed false results in the first step of identification, GC × GC-HRTOFMS with T-SEN provided accurate quantification of compounds in addition to correct identification in the crude sediment sample. The differences between the values measured by GC × GC-HRTOFMS with T-SEN and the certified values for the sediment as a certified reference material ranged from 7.3 to 36.9 % for compounds with concentrations above the limit of quantification. Except for the case of co-elution, false positives/negatives were not observed. GC × GC-HRTOFMS in combination with the tool T-SEN provided rapid and accurate screening in this demonstration, and represents a powerful new approach as post-screening in GC × GC-MS which expands the potential for comprehensive analysis.

243

Diagnostic derivatization: A novel approach to identify (aromatic) amines in environmental matrices

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The presence of aromatic amines in the environment has been in the focus of research, as many of these compounds are known or suspected mutagens and carcinogens. To facilitate the detection of aromatic amines in complex environmental samples by LC-high resolution mass spectrometry, a diagnostic derivatization method to label (in an ideal case) all aromatic amines in complex environmental matrices was developed and evaluated. About 40 compounds containing one or more aromatic or aliphatic amino groups were analyzed for method development. 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) was selected as the labeling reagent due to its high reactivity with both primary and secondary amines and its very low ionization by ESI in positive mode. As a post-column on-line derivatization did not result in sufficient yields of derivatives, our study focused on pre-column derivatization in vials prior to LC-HRMS analysis. Two strategies were tested for the detection of derivatized amines: (i) a search of peaks present in the derivatized, but not the original sample in full scan chromatograms, followed by a search for the amine precursor compound in the original sample; (ii) pseudo-neutral loss scans of the derivatized samples based on in-source fragmentation (LTQ Orbitrap) and all-ion HCD fragmentation (Q-Exactive) and search for diagnostic neutral losses from the derivative. Most of the selected aromatic amines but also the aliphatic ones result a derivative except some sterically hindered compounds and some heterocyclic amines. The reaction yield was always below 90% enabling us to detect both the analyte and the derivative in the derivatized samples for higher peak intensities. A common neutral loss of two –OH groups (m/z 17 and 34) and in some cases a subsequent –NO loss (m/z 64) were observed for most of the compounds. Chromatogram deconvolution was executed by the software MZmine and the peak lists with intensity, retention time and accurate mass were further processed by the R-Nontarget package. The full scan peak lists were used to find matching peaks of analyte and derivative. This approach performed successfully to detect derivatives and corresponding precursor compounds and allowed the detection of aliphatic and aromatic amines in environmental samples. Keywords: Diagnostic derivatization, aromatic amines, high-resolution mass spectrometry

244

Integrated Accessible Computational Strategies to Identify Emerging Environmental Pollutants using HR-MS/MS

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The recent developments in liquid chromatographic techniques coupled with high resolution mass spectrometry (LC-HRMS/MS) to capture the more polar range of environmental contaminants has resulted in an explosion in the number of target and non-target compounds for consideration in environmental analysis. Most instruments now provide data acquisition methods that allow the screening of suspect and non-target compounds post acquisition, while many emerging compounds that are of high environmental relevance have structural characteristics (substructures) that can be used to streamline identification efforts. However, no automated strategies exist to streamline the identification efforts in the environmental context. Thus, integrated computational strategies for identifying emerging pollutants on the basis of HR-MS/MS data were developed and tested. R packages were used to extract high resolution isotope, adduct and homologue information (package “non-target”) as well as the MS/MS information (“RMassBank”) from the data. MOLGEN-MS/MS was used to calculate molecular formulas, while MetFusion was used to retrieve candidate structures using ChemSpider and rank them according to *in silico* fragmentation and spectral information from MassBank. MetFusion was extended to additionally restrict the compound database search by substructures. MetFusion was first evaluated on known environmental target compounds, from standard measurements and samples. Then the integrated strategy was successfully applied to the identification of transformation products of benzotriazoles and on non-target compounds detected in wastewater effluents from Switzerland. It is clear that not all identification efforts can end in complete success with confirmation via reference standards. Thus, this work also involved the assessment of the confidence in identification efforts, ranging from a “non-target mass of interest” (Level 6) through to “Confirmed identification” (Level 1). An appropriate communication of the identification confidence is necessary to enhance the exchange of potential emerging compounds between environmental institutes, for example via the upload of MS/MS spectra of tentative and unknown compounds to MassBank.

245

Carbamazepine and its metabolites in wastewater, surface water and drinking water: Occurrences, potential risks and analytical pitfalls

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Carbamazepine (CBZ) is a widely used anticonvulsant pharmaceutical that is known to be very persistent in the aquatic environment. In this work, we comprehensively followed the fate of its main metabolites in wastewater, surface water and drinking water. The occurrence of the parent compound and its metabolites was investigated in a total of 104 water samples from Germany and Portugal by liquid-chromatography tandem mass spectrometry. The five metabolites 10,11-dihydro-10,11-dihydroxy-CBZ (DiOH-CBZ), 10,11-dihydro-10-hydroxy-CBZ (10-OH-CBZ), 10,11-epoxy-CBZ, 2-hydroxy-CBZ and 3-hydroxy-CBZ were very persistent with little to no removal during wastewater treatment. The highest concentrations were found in wastewater, with up to 4.8 and 1.1 μg/L for DiOH-CBZ and 10-OH-CBZ, respectively. In drinking water, CBZ and DiOH-CBZ could be quantified at average concentrations of 0.10 and 0.16 μg/L, respectively. Furthermore, the related pharmaceutical oxcarbazepine and the metabolites 9-hydroxymethyl-10-carbamoylacridan, 1-hydroxy-CBZ (1-OH-CBZ) and 4-hydroxy-CBZ (4-OH-CBZ) were detected in wastewater and surface water. Explicit care was taken to achieve a good chromatographic separation of the numerous isomers that were difficult to distinguish by mass spectrometry alone. Using a phenylether HPLC column combined with high resolution mass spectrometry and hydrogen-deuterium exchange, 1-OH-CBZ and 4-OH-CBZ were identified in the environment for the first time in this work. A preliminary risk assessment for CBZ revealed potential risks for the parent compound, whose measured environmental concentration level exceeded the predicted no-effect concentration of 0.5 μg/L for 37% of the surface water samples. Similar concentration levels of some metabolites suggest that risks for them cannot be excluded.

246

Investigation of micropollutants and transformation products from a waste water treatment plant with full scale ozonation using LC-HRMS

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Due to the extensive use of industrial chemicals, pesticides, personal care products and pharmaceuticals, many anthropogenic organic micropollutants and their biological metabolites have become ubiquitously detectable in the aquatic environment. The emission of these substances into the aquatic environment occurs through both direct and indirect discharge into the sewage in combination with insufficient biological or chemical degradation efficiency of conventional municipal waste water treatment plants (WWTPs). The impact of environmentally relevant micropollutants on human health and aquatic organisms has been studied not comprehensive enough; however, within the implementation of the EU Water Framework Directive it is increasingly important. Actually many research projects are focused to determin micropollutants and evaluate different elimination techniques. Ozonation as an advanced treatment step in WWTP is a relatively new approach to reduce the emission of such substances. However, using economically efficient operation conditions the oxidation generally does not result in a complete mineralization of organic substances but rather leads to partially oxidized transformation products. In North-Rhine-Westphalia the ozonation was implemented on a large scale and tested in practical operation. Against this background, samples from the influent, effluent and after ozonation from the WWTP in Duisburg-Vierlinden were picked up and analyzed using liquid chromatography high-resolution mass spectrometry (LC-HRMS). The data investigation was focused on occurrence of suspected and non-target micropollutants, biological metabolites and transformation resulting from the oxidation. For the suspected substances, a comparison of this data has been conducted with a custom-made database for micropollutants, metabolites and transformation products. The data evaluation for the non-target screening approach followed by a principal component analysis (PCA) and *in silico* methods for metabolism, transformation reactions and fragmentation pattern. The combination of a database screening approach for suspected substances and *in silico* methods to identify substances in the non-target screening analysis offers the opportunity to detected a large scale of micropollutans and their metabolites and transformation products in the waste water treatment process. Detailed results will be presented.

Ecological Consequences of Exposure to Pharmaceuticals: From the Laboratory to the Field (II)

247

Ecotoxicological assessment of antibiotics: state-of-the-art and needs for improvement

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Current environmental risk assessment (ERA) of antibiotics seek to protect the biodiversity and ecological functioning of ecosystems. However, ecosystem protection goals are only vaguely described in current guidelines for regulatory risk assessment for human and veterinary pharmaceuticals. Here we critically review the ecotoxicological assessment of antibiotics as related to ERA. Special attention is paid to environments comprising primary recipients of antibiotic residues (e.g. agricultural soils, aquaculture, and waste water impacted environments), and we focus on microbial communities as bacteria comprise the primary targets for antibiotics. We initially discuss the need for more specific protection goals for ERA of antibiotics, and key ecosystem services provided by microbes including their key drivers (i.e. ecosystem service-providing units such as taxa or functional groups) are identified. We propose that ERA should be designed to protect microbial community functions, microbial ‘species’ richness, and antibiotic susceptibility of environmental bacteria. We subsequently review the methods currently in use for ecotoxicological assessment of new human, agricultural and veterinary medicinal products and discuss the need for the development and inclusion of improved microbial test methods that allow for targeted protection of the ecosystem services provided by microbes. Specifically, we discuss the appropriateness of different ecotoxicological endpoints (i.e. community function, structure or tolerance) for ERA. Ecotoxicological endpoints focused on ‘higher’ trophic levels are also emphasized due to their high priority for ERA. Hence, antibiotics may adversely affect non-target organisms such as selectively grazing bacterivores or higher-order consumers indirectly through trophic cascades. We also discuss the accumulative risk of mixtures of antibiotics as there is a need to address the potential for additive or synergistic impacts of mixtures of antibiotics on bacterial communities and higher trophic levels. Finally, we discuss the effects of sub-inhibitory concentrations of antibiotics, which may lead to disruption of microbial signaling thereby potentially affecting some ecosystem services provided by microorganisms. Major knowledge gaps will be highlighted in order to propose promising areas for future research that will enable a more solid foundation for assessing risks associated with antibiotic residues in the environment. The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

248

A multi-species approach to evaluate the acute and chronic toxicity of carbamazepine, ibuprofen and propranolol spiked in sediment samples.

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In a way to bring information about suitable tools for ERA of pharmaceutical products, a battery of conventional bioassays was applied using marine organisms exposed to sediment samples spiked with pharmaceutical products: carbamazepine (CBZ), ibuprofen (IBU) and propranolol (PRO): (500$\mu g \cdot L^{-1}$, 50$\mu g \cdot L^{-1}$, 5$\mu g \cdot L^{-1}$, 0.5$\mu g \cdot L^{-1}$, 0.05$\mu g \cdot L^{-1}$), including the environmental concentrations (underlined). Microalgae growth inhibition of *I. galbana* and *T. chuii* was significant different to the control for CBZ (50$\mu g \cdot L^{-1}$, 5$\mu g \cdot L^{-1}$, 0.5$\mu g \cdot L^{-1}$, 0.05$\mu g \cdot L^{-1}$) and PRO (50$\mu g \cdot L^{-1}$ only for *T. chuii*) ($p < 0.05$). Concerning the sperm and embryogenesis assays of sea-urchin *P. lividus*, embryogenesis success showed significant effect after exposure to CBZ (500$\mu g \cdot L^{-1}$, 5$\mu g \cdot L^{-1}$, 0.05$\mu g \cdot L^{-1}$), IBU (500$\mu g \cdot L^{-1}$, 50$\mu g \cdot L^{-1}$, 0.5$\mu g \cdot L^{-1}$) and PRO (all) ($p < 0.05$). Microtox® Basic Test represented hormesis for elutriate samples. The ranking of EC_{50} for the acute toxicity observed was: CBZ>IBU>PROP for Microtox® BSPT. After 15-days of polychaetes exposure, chronic responses were determined through biomarkers of exposure and effect . The activity of the Phase I of the metabolism was activated for PRO (increase EROD and decrease DBF) and IBU (decrease DBF for 500$\mu g \cdot L^{-1}$ and 0.5$\mu g \cdot L^{-1}$) ($p < 0.05$). Phase II (GST) was activated for CBZ (increase for 500$\mu g \cdot L^{-1}$ and 50$\mu g \cdot L^{-1}$, and decrease for 0.05$\mu g \cdot L^{-1}$) and PRO (decrease for 500$\mu g \cdot L^{-1}$, 5$\mu g \cdot$L-1, 0.5$\mu g \cdot L^{-1}$) ($p < 0.05$). Antioxidantenzymes showed significant increase compared to the control for 0.05$\mu g \cdot L^{-1}$CBZ (GPX) ($p < 0.05$). Lipid peroxidation significantly increased compared to the control for CBZ (500$\mu g \cdot L^{-1}$), IBU (5$\mu g \cdot L^{-1}$) and PRO (5$\mu g \cdot L^{-1}$, 0.5$\mu g \cdot L^{-1}$, 0.05$\mu g \cdot L^{-1}$) ($p < 0.05$). Genetic damage significantly increased compared to the control for IBU (500$\mu g \cdot L^{-1}$,5$\mu g \cdot L^{-1}$) and significantly decreased for PRO (50$\mu g \cdot L^{-1}$) ($p < 0.05$). Described results showed the bioavailability of these substances in marine sediments and the confirmation of the metabolism of detoxification in benthic invertebrates.

249

Investigating various modes of action and potential adverse outcomes of pharmaceuticals in marine mussels

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Pharmaceutical concentrations in marine coastal waters are in general very low, however dilution cannot be considered a safety factor. Indeed, pharmaceuticals are designed to interact with specific biological targets and produce effects at the lowest possible doses. These effects are not necessarily revealed by acute or chronic toxicity tests on standard organisms, and the need for new approaches is underlined. The application of the Mode of Action (MOA) approach may provide a substantial contribution to the understanding of subtle, specific effects of pharmaceuticals on non-target organisms, since the evolutionary conservation of molecular targets in a given species potentially increases the risk of effects of bioactive compounds. We explored several MOA-relevant endpoints and biomarkers responses to investigate possible adverse outcome pathways of fluoxetine (FX) and propranolol (PROP) on the Mediterranean mussel, *Mytilus galloprovincialis*. Mussels were exposed for 7 days to FX or PROP (0.3 - 300 ng/L range) or to the combination FX+PROP (each at 0.3 ng/L). MOA-relevant endpoints, including cAMP levels and PKA activities, were analysed in digestive gland. Biomarkers of lysosome functionality, and catalase and GST activities were measured in digestive gland, lysosome membrane stability (LMS) in haemocytes. Both FX and PROP decreased cAMP levels and PKA activities, in agreement with the inhibition of the cAMP pathway through 5-HT1 receptors. The presence of 5-HT1 receptors in mussels and modulation of their mRNA levels by 5-HT and FX was confirmed by real time PCR. The main effects of FX+PROP on cAMP levels and PKA activity were ascribed to PROP, supporting a prevalent adrenergic control in digestive gland. Both FX and PROP caused a dose-dependent reduction of LMS in haemocytes, at all concentrations tested. LF, NL, Lys/Cyt ratio and CAT activity were significantly affected, although the effects did not appear dose-dependent. FX+PROP lowered LMS stability, and increased Lys/Cyt ratio, LF and NL accumulation. In conclusion, environmental pharmaceuticals may be highly active on wildlife possessing specific molecular targets, therefore MOAs should be explored to reveal adverse outcome pathways of pharmaceuticals and interactions occurring between them. The use of biomarkers demonstrated drug effects at environmental concentrations. Approaches based on MOAs and biomarkers are suggested as a priority task for pharmaceutical risk assessment.

250

Fate and effects of pharmaceutical antibiotics differ among single substances and mixtures

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It is the rule rather than the exemption that livestock animals are medicated not only with a single pharmaceutical antibiotic but combinations of antibiotics are administered simultaneously or consecutively. As a consequence, excreta stored in tanks or lagoons contain mixtures of several antibiotics that are subsequently spread as fertilizer on agricultural soils. However, knowledge on the fate and effects of such mixtures is scarce. In laboratory experiments, the fate and effects of mixtures of antibiotics was investigated compared to that of single substances. To this end different tetracycline and sulfonamide antibiotics as well as trimethoprim were tested in selected combinations. Sorption and dissipation was determined in batch and microcosm experiments; the iron(III)-reduction test was used to determine dose-response curves. Competitive sorption was found among antibiotics of one structural class and between structural classes, resulting in significantly higher mobility of the antibiotics. Also, the dissipation rate in microbially active as well as in sterile soil was retarded in the presence of antibiotic mixtures. While concentration additivity (CA) was found for tetracyclines, mixtures of two sulfonamides and of a sulfonamide with trimethoprim and tetracyclines, respectively, showed increased adverse effects on soil microorganisms. These were best described by the model of independent action (IA). The isobologram of the combined action of chlortetracycline and sulfadiazine showed clear superadditivity of effects (Loewe synergism). It is concluded that the existing knowledge on ecotoxicity of antibiotics is insufficient to assess the existing contaminations with mixtures of antibiotics.

251

Dilute concentrations of an anxiolytic drug alters behaviour and species interactions of wild fish

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Environmental pollution by pharmaceuticals is increasingly recognized as a major global threat to aquatic ecosystems[1]. Many pharmaceuticals enter waterways via treated wastewater effluents [2-6] and remains biochemically active in aquatic systems [4,7]. Several ecotoxicological studies have been made but generally, little is known of the ecological effects of pharmaceuticals [4,8]. Here I present data from a laboratory exposure-study of wild caught fish showing that a benzodiazepine anxiolytic drug (*Oxazepam*) alters ecologically important behaviours (e.g. activity and sociality), feeding rate and predation risk of wild European perch *(Perca fluviatilis)*at concentrations close to those encountered in effluent influenced surface waters. Individuals exposed to water with dilute drug concentrations (1.2 $\mu g\ l^{-1}$) exhibited increased activity, reduced sociality, higher feeding rate and increased predation risk. In aquatic environments, predation is an important driver of species numbers, community composition, and productivity [9,10]. As such, the results show that anxiolytic drugs in surface waters can profoundly alter both species interactions and animal behaviors that are known to have ecological and evolutionary consequences.

252

The striking sensitivity of invertebrates to antidepressants

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Antidepressants are among the most commonly detected human pharmaceuticals in the aquatic environment. Since their mode of action is to modulate the neurotransmitters serotonin, dopamine, and norepinephrine, aquatic invertebrates that possess transporters and receptors sensitive to activation by these pharmaceuticals are potentially affected by them. We review the various types of antidepressants, their occurrence and concentrations in aquatic environments, and the actions of neurohormones modulated by antidepressants in invertebrate models. Recent studies on the effects of antidepressants, on particularly crustacean and molluscan groups show they are susceptible to a wide variety of neuroendocrine disruption at environmentally relevant concentrations (pg-ng/L). For example molluscs show that antidepressants affect spawning and larval release in bivalves, disrupt locomotion/reduce fecundity in snails and camouflage/memory function in cephalopods. In crustaceans, antidepressants affect activity patterns, photo- and geotactic behaviour, gene expression, reproduction and development. In contrast, studies to date have primarily been finding effects in fish at considerably higher concentrations (μg/l-mg/l). We discuss possible reasons for the discrepancy in these results in relation to the "read-across" hypothesis, variation in biomarkers used, phylogenetic distance, and the affinity to different targets and differential sensitivity to receptors. We also highlight the increasing reported occurrence of non-monotonic dose responses curves, often with effects at low concentrations, but not at higher concentrations, and we suggest future experiments consider testing a broader range of concentrations. There is growing evidence to suggest antidepressants do affect aquatic invertebrates at concentrations currently found in the environment. However, providing strong evidence for effects in the field will be a tough new challenge for ecotoxicologists.

Detection and characterisation of nanomaterials in complex aqueous matrices

253

Behaviour and fate of silver and manufactured silver nanoparticles along the wastewater-biosolid exposure pathway

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Silver nanoparticles (Ag-NPs) are present in more products that any other nanoparticles, and studies using probabilistic material flow analysis have consistently shown that the major environmental release pathway for Ag-NPs is the wastwater-biosolids pathway. It is important to understand how the characteristics of Ag-NPs, in terms of core structure and surface functionality, affect their behaviour and fate along this pathway. Here, we report a comprehensive set of studies which investigated the following aspects of this release pathway: a) Stability of Ag-NPs in a simulated sewer network, including pumping stations (sewer retention time 16 hours; total experiment duration 14 days).; b) Transformations of various Ag-NPs (with different surface functionality and core composition) in municipal wastewater sludge and during anaerobic sludge digestion; c) Speciation and lability of Ag in a collection of experimentally derived, contemporary (fresh and aged) and historical biosolids (some of which had been aged in open-air stockpiles for up to 50 years) from Australia, the USA and UK. Silver speciation was measured using X-ray absorption near edge spectroscopy (XANES) and silver lability was assessed via an isotopic dilution technique using ^{110m}Ag (E values). Various supporting parameters were measured (e.g. pH, Eh, sulfides), and we also investigated the use of a novel device incorporating immobilised Ag-NP on plasma polymerised surfaces for assessing their transformation in situ. The results reported here clearly demonstrate that: (a) the speciation of Ag in the wastewater-biosolid exposure pathway is largely independent of the form released from urban sources in wastewater (e.g. ionic, Ag/AgCl-NPs); (b) Ag bound to reduced S is the dominant form of Ag along this exposure pathway; and (c) Ag sulfides persist as the dominant species in biosolids even after prolonged stockpiling, and Ag lability is accordingly very low.

254

Fate dynamics of silver nanoparticles in sewage treatment studied with FAST single-particle ICP-MS

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FAST spICP-MS is a new development of conventional single particle ICP-MS (spICP-MS) where ion clouds originating from nanoparticles (NP) in suspension are measured using very short subsequent acquisition times, so that multiple datapoints per particle event are obtained. The benefits for conventional spICP-MS have been shown for acquiring number-based particle size distributions (PSD) of NP in complex aqueous samples. The current work progresses further by presenting an application of the FAST spICP-MS methodology to particularly challenging samples of Ag NP spiked into a wastewater treatment plant (WWTP) microcosm. A mixture of samples taken freshly from a real WWTP in the Gothenburg area was used for the sake of realism. Three stages (anaerobic, aerobic and settling) of a WWTP were thus simulated and the PSD of spiked AgNP was measured using FAST spICP-MS as a function of time, allowing to monitor the dynamics of AgNP fate in WWTP such as aggregation, dissolution and sedimentation. Benefits of FAST spICP-MS such as fewer multiple and incomplete particle events and the size and particle number concentration detection limit are discussed. The results also demonstrate the fast dissolution and sedimentation AgNP undergo in a WWTP, explaining the high partitioning of AgNP to biosolids.

255

Microsecond spICP-MS and its application to environmental samples

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The potential release of engineered nanomaterials into the environment has required the development of more sophisticated, selective, and sensitive analytical techniques to detect and characterize these materials in complex environmental matrices. In recent years, single particle ICP-MS (spICP-MS) has been used as a sensitive, high throughput technique for the detection and sizing of nanomaterials at environmentally relevant concentrations (ng/L). This is accomplished by using short dwell times (milliseconds) to record a discrete pulse of intensity resulting from a nanoparticle event that is consequently much larger than the background. However, this technique has been hampered in cases of high particle number concentration and elevated dissolved backgrounds. The work presented here shows that these obstacles are overcome by reducing the dwell time to microseconds. In this range, the particle is presented as a distribution of pulse intensity relating to temporal detection of the particle ion cloud as it reaches the detector. This allows for great improvements in particle resolution to be made, allowing for analysis of samples with higher dissolved backgrounds and high particle number concentrations. In addition, as a nanoparticle detection event occurs over the span of a couple hundred microseconds, it is now possible to detect two elements within a single nanoparticle. By switching between two monitored isotopes, elemental and isotopic ratios can be determined on a particle-by-particle basis. This enables for the possibility of an analytical method to differentiate between naturally occurring nanoparticles, which may have a natural abundance of two elements, and their elementally/isotopically enriched engineered analogues. In addition, more complex nanomaterials with a core-shell structure can be characterized with these improvements. The addition of microsecond spICP-MS to the growing number of nano-analytical techniques should provide a new approach to overcoming some of the challenges in the detection of ENPs in complex matrices.

256

Evaluating Bioaccumulation of Silver and Gold Nanoparticles Using spICPMS-Enabled Tissue Extraction and Analysis

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Expanded use of engineered nanoparticles (ENPs) in consumer products increases potential for environmental release and unintended biological exposures. As a result, measurement techniques are needed to accurately quantify ENP size, mass, and particle number distributions in biological matrices. This work combines single particle inductively-coupled plasma mass spectrometry (spICPMS) with tissue extraction to quantify and characterize metallic ENPs in environmentally relevant biological tissues for the first time. ENPs were extracted from tissues via alkaline digestion using tetramethylammonium hydroxide (TMAH). Method development was performed using ground beef and was verified in *Daphnia magna* and *Lumbriculus variegatus*. ENPs investigated include 100 and 60 nm Au and Ag stabilized by polyvynylpyrrolidone (PVP). Mass- and number-based recovery of spiked Au and Ag ENPs was high (83-121%) from all tissues tested. Additional experiments suggested ENP mixtures (60 and 100 nm Ag ENPs) could be extracted and quantitatively analyzed. Biological exposures were also conducted to verify the applicability of the method for aquatic organisms. Size distributions and particle number concentrations were determined for ENPs extracted from *D. magna* exposed to 98 μg/L 100 nm Au and 4.8 μg/L 100 nm Ag ENPs The *D. magna* nanoparticulate body burden for Au uptake was 613 ± 230 $\mu g/kg_{ww}$, while the measured nanoparticulate body burden for *D. magna* exposed to Ag ENPs was 59 ± 52 $\mu g/kg_{ww}$. Notably, the particle size distributions determined from *D. magna* tissues suggested minimal shifts in the size distributions of ENPs accumulated, as compared to the exposure media.

257

Visualisation of silver nanoparticle uptake by Laser Ablation - Inductively Coupled Plasma Mass Spectrometry

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According to the unique size, surface area, and reactivity of nanoparticles (NP) an improvement in the toxicological testing, the uptake quantification and risk assessment is required. As a consequence, the testing and exposure strategies for nanoparticles have to be adjusted. Beside the observation of biological effects of nanoparticles *in vivo* and *in vitro*, there is a need of adequate analytical detection and quantification methods. The quantification of uptake of metal containing nanoparticles by environmental organisms or cells can be performed by digestion of the organic material to dissolve the particles, followed by Inductively Coupled Plasma Mass Spectrometry (ICP MS) as a robust element detection method. However, this approach only provides information on the total amount of NP taken up, it is not possible to distinguish between particles or a dissolved porportion. Additionally, the connection to the localisation of particles inside the biological tissues and the correlation with mechanisms leading to the occurrence of toxic effects is still missing. Such information can be obtained by imaging techniques. Up to now, most imaging methods utilize the modification of physical and chemical particle properties, e.g. by fluorescence labelling, to make particles visible. In this study, a reliable and quantitative imaging method was developed by coupling a Laser Ablation system with an ICP MS (LA-ICP MS) to enhance the knowledge of how nanoparticles are taken up and how they are distributed in environmental organisms (e.g. *Danio rerio*, *Daphnia magna*). With this method quantitative data on NP uptake can be gathered and particles were detected without the need for (fluorescence) labelling. By using this imaging method the interaction between the processes of particle uptake, the bioaccumulation, and the occurrence of toxic effects can be understood in a more detailed manner. The benefits of this approach will be shown with respect to silver nanoparticle (AgNP) uptake.

258

Detection and quantification of nanoparticles in aqueous matrices by means of field flow fractionation and mass spectrometry

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Because of their chemical and electrical properties, nanoparticles such as fullerenes or Ag have been widely applied in personal care products, drug delivery systems and solar cells. The fate of manufactured nanoparticles have been increasingly studied because of their potential risks to the environment and human health. In commercial applications such as organic photovoltaic cells, derivatised fullerenes are used to modify their solubility and electronic properties. Silver nanoparticles are being employed in health care and consumer products. Aim of the present work is to develop and optimise a method that allows the detection, size determination and quantification of fullerenes (and their clusters) in aqueous matrices by means of Orbitrap MS and FFF, both stand-alone and combined. For the size determination a MALS detector is used. For the extraction of fullerenes from different aqueous matrices SPE was applied. Various materials were tested and recoveries determined. Additionally, the influence of other parameters (ionic strength, humic acid) was assessed/evaluated.The size distribution of fullerenes in water was determined with FFF coupled to a MALS detector. After optimization of the separation method, the FFF was linked to the Orbitrap to allow quantification of the different size fractions. Different SPE column materials (C18, C18e, C8, CN) were tested, and recoveries appeared to be the highest for the C18-material. Recoveries were improved by adding NaCl to the water during extraction and higher concentrations of humic acid had an negative effect on the recovery. Very low limits of detection (LOD) values were obtained for all compounds with this method, ranging from 0.17 ng/L for [70]PCBM to 0.28 ng/L for C60, and subsequent limit of quantitation (LOQ) values of 0.57−0.91 ng/L. Recoveries for the fullerenes were on average 120% in ultrapure and drinking water. Recoveries appeared to be lower, but still acceptable (e.g., >78%), in surface water. The FFF/MALS-analysis of aqueous samples of fullerenes showed that the sizes of the aggregates range between 4 nm to 200 nm. Fractograms show that the void peak and the first fullerene clusters are clearly separated. Analysis by FFF coupled to the Orbitrap confirmed that the particles observed are fullerene (clusters).

Bioavailablity and effects of metals and metal mixtures (II)

259

Mixed stress? Growth and bioaccumulation of freshwater algae Pseudokirchneriella subcapitata under combined zinc and phosphate supply

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In the past, researches have observed P-metal interactions on the growth of algae while inconsistent results were found across those studies. We propose that probably due to methodologies adopted in different experiments. The objectives of this work are to investigate Zn×P interactions (growth and Zn-P uptake) under steady state supplies of P. A constant P supply in exponentially growing algal cultures was obtained by exponential dosing of P at various exponents. The algae were grown at semi-steady state under 3 different constant growth rates (0.8 day^{-1},1.15 day^{-1} and 1.45 day^{-1}). Toxicity test with 7 factorial Zn concentrations from 13 μg/L to 256 μg/L was afterwards performed. Cell P as well Zn concentration was measured by acid dissolution followed by the analysis of the ICP-MS. It was observed that the cell P concentration increases with Zn supply. At the same time, the cell Zn exhibited an increasing trend with P supply. P-Zn precipitation was suspected to occur inside the cell. Furthermore a 3-D kinetic model was fitted for the intrinsic interactions between P and Zn toxicity. The growth rate μ was depicted as a function of cell P concentration and external Zn concentration and the entire process of the toxicity experiments were stimulated. The model was optimized by non-linear regression.

260

Development of a chronic metal mixture bioavailability model for Ni-Zn toxicity to Daphnia magna based on the independent action model

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Risk assessment of metals is generally based on ecotoxicity test results conducted with individual metals. However, organisms in the environment are mostly exposed to mixtures of metals. Both non-interaction, synergism and antagonism have been observed in metal mixture assays. This uncertainty about metal mixture interactions hinders the development of a metal mixture toxicity model to predict the toxicity of metal mixtures in the environment and therefore also the incorporation of metal mixture toxicity in risk assessment. Furthermore, most metal mixture studies consider acute toxicity, while chronic effects of metal mixtures are rarely considered. Moreover, although metals have often a dissimilar mode of action, the Concentration Addition (CA) model is commonly used to describe metal mixtures. In this study, we studied binary metal mixture interactions in a Ni-Zn mixture in two independent chronic reproduction tests with *Daphnia magna*. As these metals have (at least partly) dissimilar known modes of action, we used the Independent Action (IA) model to analyze metal mixture interactions. Possible metal interactions were assessed with the IA model by evaluating if the concentration response curve on the basis of Me^{2+} activity of one metal changes in the presence of the other metal. If the EC50, expressed as Me^{2+} activity; of the first metal is significantly higher (lower) in the presence of the second metal, the metals are interacting antagonistic (synergistic). Both Ni and Zn were shown to interact synergistically at high concentrations (based on Me^{2+} in the solution) for *D. magna*. A Metal Mixture bioavailability model was developed by combining the existing bioavailability models for individual Ni and Zn toxicity with the IA model, and by integrating a model term expressing the synergistic interaction. This model showed reasonable predictive capacity. These models can be valuable in future risk assessment processes for metals.

261

Toxicity of binary mixtures of nickel, copper, cadmium and zinc to Daphnia magna

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Although metals are regulated on a metal-by-metal basis in most jurisdictions, metals usually occur in mixtures instead of alone in water. However, the toxicity of metal mixtures is difficult to predict accurately. As part of a project to provide mixture-toxicity data for development of multi-metal toxicity models, we tested the toxicity of binary Cd-Ni, Ni-Cu, and Ni-Zn mixtures. To analyze the interactions of these metals, *Daphnia magna* neonates were exposed to the metals alone and in binary combinations in standard 48-h lethality tests. For each combination of metals in the binary mixtures, one metal was held constant at a specified concentration while the second metal was varied through a series that ranged from nonlethal to lethal concentrations; then the roles of the two metals were reversed in a separate series of tests. Based on dissolved-metal concentrations, the toxicity of Ni-Cd mixtures was less-than-additive (i.e., less toxicity than predicted from single-metal tests); the toxicity of Ni-Cu mixtures was more-than-additive (i.e., greater toxicity than predicted from single-metal tests); and in preliminary results, the toxicity of Ni-Zn mixtures was additive or slightly less-than-additive. These results provide evidence for the dominance of competition of metals for complexation to biological ligands or to dissolved organic matter, depending on the metal combination in question.

262

Mixture toxicity and interactions of copper and zinc to barley in three different soils

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Soil metal contamination mostly occurs as a mixture of different metals, rather than as a single metal. Risk-evaluation of metal contamination in soil, however, is almost always based on the effects of single metals. This is only justified if the exposure to mixtures does not bear the risk of an increased toxicity relative to the toxicity at single exposure. Therefore, there is a need to understand how metals act together in producing combination effects. Metals may interact in mixtures and these interactions in soil may partly be related to interactions on their bioavailability. It is postulated that metals may act synergistically because of the limited specific sorption sites that can reduce the metal bioavailability. Hence, mixture toxicity and interactions of copper (Cu) and zinc (Zn) to barley are assessed in three soils, differing in cation exchange capacity (2, 7 and 85 $cmol_c$/kg). The interactions will be assessed based on both total metal concentrations and free metal ion concentrations. To model mixture toxicity, two basic models: the concentration-addition (CA) model and independent action (IA) model will be used and compared. The hypothesis is that synergistic interactions will be observed based on total concentrations with the IA model as reference model and that perfect addition will be observed with the CA model as reference model. In addition, we postulate that the interactions are most pronounced in the soil with the highest CEC. These synergistic interactions with the IA model as reference model can be explained by competition effects between the metals for binding to soil binding sites. Metal toxicity depends on the free metal ion activity. When a second metal is added to a soil, it will (partly) bind to the binding sites, thereby releasing the previously bound first metal, increasing its free ion activity and toxicity. It is expected that when mixture toxicity is modeled based on free metal ion concentrations, perfect IA will be observed. Preliminary results for two soils (CEC 7 and 85 $cmol_c$/kg) confirm perfect CA based on total metal concentrations. Significant synergistic interactions between Cu and Zn, with the IA model as reference model, however, are only observed in the potting soil (highest CEC). The EC50 of Cu decreases ($p< 0.05$) from 474 to 296 mg Cu/kg soil, when 1100 mg/kg Zn is added to the soil compared to the no Zn amended soil. The combination

toxicity data will be presented at the meeting.

263

Metal accumulations in diffusive gradients in thin films (DGT). Mixture and low ionic strength effects.

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DGT [1] is a powerful technique for in situ measurement of metal fluxes. It consists of a resin immobilised and embedded in a gel (the resin disc) that strongly binds the metal ions that arrive to it after crossing a diffusive gel disc. The metal complexes able to diffuse through the diffusive gel also penetrate into the resin layer [2,3]. This penetration of complexes leads to an important contribution to the total metal accumulation. Additionally, the influence of the composition of the mixture of ligands is reduced to the effects acting in the gel domain [4]. In the presentation, results showing the influence of the mixture composition on the metal accumulation will be presented together with the impact of low ionic strength. The penetration of complexes into the resin disc can be strongly influenced by an electrostatic effect derived from the electrical charges of the resin beads. For decreasing ionic strengths, negatively charged complexes will experience stronger electrostatic exclusion leading to a reduction of the accumulated mass, while positively charged complexes will contribute to enhanced metal accumulations. Experimental evidences for this behaviour will be provided as Ni, Cd and Co accumulations in presence of nitrilotriacetic acid (NTA) or ethylenediamine (Etdiam) which form negatively and positively charged complexes, respectively. A simple electrostatic model can quantitatively explain the results. Simple expressions for the metal accumulation can be obtained and fitted to the experimental accumulations leading to values of the Boltzmann factors which can be used in other systems. All these effects were not previously described and are extremely important for a correct interpretation of DGT measurements in pristine waters [5]. Likewise, the increased kinetic dissociation constant in the resin domain (in comparison to the Eigen value in the gel domain) is an important phenomenon that lends support to the interpretation (in a large number of applications reported in the literature) of DGT probing mostly the labile concentrations. References: [1] W.Davison, H.Zhang, Nature 367 (1994) 546. [2] S.Mongin, R.Uribe, J.Puy, et al. Environ. Sci. Technol. 45 (2011) 4869. [3] J.Puy, R.Uribe, S.Mongin, et al. J. Phys. Chem. A 116 (2012) 6564. [4] R.Uribe, J.Puy, J.Cecilia, et al. Phys. Chem. Chem. Phys. 15 (2013) 11349. [5] M.Alfaro-De la Torre, P.Y.Beaulieu, A.Tessier, Anal. Chim. Acta 418 (2000) 53.

264

A toxicokinetics approach to assess bioavailability of copper, chromium and arsenic to earthworms in contaminated soils

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In Finland there are more than 20,000 contaminated sites including around 200 sites used for wood salt impregnation, often using a copper-chromium-arsenic (CCA) mixture. The remediation of those sites requires insight into the potential risk of the contamiation, which asks for determining bioavailability of the contaminants. In this study soils were taken from an old wood impregnation site, from four different spots with high, medium, and low pollution level, and a control (C) to obtain a gradient of CCA contamination. An uptake-elimination kinetics experiment following OECD guideline 317, using the earthworm *Eisenia andrei*,was performed to assess bioavailability of the metals, which was related to total, water and 0.01 M $CaCl_2$ extractable copper, chromium and arsenic concentrations in the soils. In the low contaminated soil, containing 12.5 mg Cr/kg dry soil, 5.1 mg Cu/kg and 10.1 mg As/kg, no uptake of copper, chromium and arsenic was seen, with earthworm concentrations remaining more or less constant at approximately 7.7, 0.9 and 22 mg/kg body weight, respectively throughout the uptake and elimination phases. The medium and high contaminated soils contained similar Cr (1477-1592 mg/kg) and Cu (642-791 mg/kg) levels, while As concentration was highest in the high contamined soil (850 versu 2812 mg/kg). In both soils, copper and chromium showed very fast uptake and elimination kinetics. For copper, uptake rate constants (k1) were 0.16-0.19 kg soil/kg earthworm/day and elimination rate constants (k2) 2.2-2.4 per day. For chromium uptake and elimination were so fast that it was hard to obtain reliable k1 and k2 values. For both metals, steady level was reached within a day and after transfer of the earthworms to clean soil they reached the background level also within one day. Arsenic showed very slow uptake and elimination kinetics in the medium and high contaminated soils. Steady state was not reached within 21 days of exposure. For arsenic, k1 values were 0.0065-0.011 kg soil/kg earthworm/day and k2 was 0.006-0.012 per day. From these values, a bioaccumulation factor (BAF) of 0.55-1.78 was estimated for arsenic bioaccumulation in earthworms. The results show that in the low contaminated Hartola soils, the CCA metals have a low bioavailability. In the medium and high contaminated soils, however, all metals are bioavailable and taken up by earthworms. It seems the risk of CCA-contaminated soils near Hartola is mainly in the high bioavailability of arsenic.

Modelling of chemical fate and exposure in a regulatory context (II)

265

EFSA Guidance Document on clustering and ranking of emissions of plant protection products from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments

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EFSA was asked by the Commission (DG SANCO) to draft an EFSA Guidance Document [3]. This guidance is intended for the risk assessment of plant protection products (PPPs) active substances and their transformation products (metabolites) when emitted from covered crop structures. Guidance is provided for when the same methodology as for open field can be used and be considered representative or conservative, and when special approaches are more appropriate.

266

Progress in regulatory degradation kinetics

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This contribution discusses the potentials and shows examples of using the single first-order reversible binding (SFORB) kinetic model for parent and metabolites. Secondly, an elegant method for the generation of plausible confidence intervals for rate constants and sets of formation fractions based on internal parameter transformations is presented. Definition of kinetic models, forward simulation (prediction) and fitting was performed using the R package mkin.\n While the single first-order (SFO) kinetic model is a one-box model, the dual first-order in parallel (DFOP) model and the SFORB model are best described as two-box models. In spit of the two forms of the parent compound occuring in these models, the pattern of metabolite formation generated from them is exactly the same. However, using the SFORB model makes it possible to use Eigenvalue based solutions, which is significantly faster than using the lsoda algorithm from the deSolve package. Benchmarking results for solving the kinetic models discussed above are given in Table 1.\n\n Model deSolve (lsoda) Eigenvalue DFOP - SFO 0.276 s not implemented SFORB - SFO 0.374 s 0.005 s *Table 1: Benchmarking results in seconds for the different model variants and solution methods* The precision of the Eigenvalue based solution is illustrated by the fact that the lsoda based solutions converge to the eigenvalue based solutions when the absolute and relative error tolerance used in calling lsoda are decreased. For the purpose of leaching simulations, the SFORB model parameters can be converted to the parameters of the kinetic sorption model implemented in the FOCUS-PEARL and FOCUS-PELMO models when linear sorption is assumed. For metabolites, a lack of fit of the SFO model due to biphasic degradation can sometimes be observed in aerobic soil degradation data, and the fits to such data can substantially be improved by using the SFORB model for these metabolites, resulting in lower χ^2 error-levels and possible leading to acceptance of a metabolite fit where the SFO fit would be rejected. The use of of internal parameter transformations for making the assumption of a normal distribution for the parameter estimates viable has been demonstrated earlier. Meanwhile, the calculation of confidence intervals on the basis of these transformations has been implemented, and practical examples, including plausible confidence intervals for formation fractions are demonstrated. \n \n \n

267

EFSA guidance for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and their transformation products in soil

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EFSA was asked by the Commission to prepare a guidance of EFSA for evaluating laboratory and field dissipation studies to obtain degradation rate parameters (DegT50 values) of active substances of plant protection products and their transformation products in soil. The EFSA guidance provides guidance for users on how to obtain *DegT50* values when performing risk assessments according to Regulation EC no 1107/2009 of the European Parliament and the Council. The aims of this guidance are: (i) to provide methods to derive the soil matrix *DegT50* from individual laboratory and field dissipation studies, (ii) explain how to determine whether the databases of *DegT50* values from laboratory and field studies can be treated as separate databases or whether they should be pooled, (iii) provide guidance on selecting the appropriate input value for use in exposure modelling. The guidance for the field dissipation studies considers two aspects. The first considers the design of tailored *DegT50* field studies. Such studies need an experimental design that aims to exclude the influence of surface processes and

leaching as far as possible. The kinetic fitting of the experimental results should be carried out following FOCUS kinetics guidance. The second aspect considers the procedure to be taken where surface processes have not been minimised. This procedure consists of decision-making flow charts to derive the most appropriate kinetic model and thus derive the *DegT50*. The underlying principle of these flow charts is that the degradation rate obtained should not include other processes than degradation within the soil matrix. The purpose of the selection of the appropriate input value for use in exposure modelling is to obtain a median *DegT50* for the population of agricultural/ horticultural field soils in the area of use of the substance. The medians of the laboratory and field *DegT50* values are estimated using a geomean estimator. The selection is based on a decision-making flow chart in which it is tested whether the *DegT50* values from the separate laboratory and field databases are statistically different. If yes, the laboratory values are discarded and the field DegT50 values are used provided that at least four field *DegT50* values are available for active substance or three for each metabolite. If no, the guidance recommends pooling all the laboratory and field *DegT50* values. Note: the content of this abstract does not necessarily represent the official views of EFSA.

268

Which role plays the scenario selection for pesticide risk assessment of soil organisms? – A case study with the vertical distribution model for Collembola FOLCAS

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This study investigated how scenario selection in exposure assessment may influence the ecological risk assessment for soil organisms. For this purpose the recently developed individual-based vertical distribution model FOLCAS - Folsomia candida Simulation - was used. FOLCAS simulates the dispersal of the soil-dwelling collembolan *Folsomia candida* in an agricultural soil column in order to investigate effects of variations in environmental parameters on population behaviour and distribution in the soil. In addition, the model is able to evaluate the influence of a pesticide application by simulating the exposure of the population as a result of their dispersal including the subsequent effects on reproduction and mortality. The environmental parameters and pesticide exposure data are derived from calculations with the numerical fate model PEARL. FOLCAS was applied to simulate different European agricultural soils in order to evaluate the effect on the dispersal of the population and therefore on the possible exposure to a pesticide under a range of conditions. In addition, different crops were simulated to elucidate the effect of crop selection on the population distribution. The off-market pesticide Lindane was used as a model substance at concentrations within the dose-response range for *F.candida*. Furthermore, recently published European scenarios for assessment of exposure of soil organisms to plant protection products were tested ("EFSA Scenarios"). The results indicate that the exposure of collembolan communities depends on the environmental parameters, the emerging vertical distribution, and the soil properties of the according scenario simulated and the population demography. First tests using the EFSA scenarios showed that winter temperatures in the scenarios are too cold to be able to establish stable populations of *Folsomia candida* given the current literature based parameterization of the model. Based on this study it can be concluded that scenario selection in exposure assessment influences the ecological risk assessment for soil organisms. Study results further elucidate the influence of crop selection by illustrating different effect expressions of pesticides on collembolan populations living in soil. Through linking exposure and effect assessment ecological modeling offers a powerful tool to identify discrepancies and subsequently to take a step toward bridging the gap between exposure and effect assessment in environmental risk assessment.

269

Importance of exposure dynamics in aquatic ecosystem risk assessment

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The goal of ecological risk assessment (ERA) is to quantify the risk that a given chemical would impair the structure and function of natural ecosystems by assessing its environmental exposure and the expected ecological aspects. However, the environmental realism, ecological relevance and methodological accuracy of the currently used exposure and effect assessment approaches have been questioned for years. For example, exposure to chemicals is generally modelled using *steady-state* approaches, which do not account for spatial and temporal variations of emissions and environmental parameters. In this context, the European Chemical Industry Council (CEFIC)-Long-range Research Initiative project “ChimERA: An integrated modelling tool for ecological risk assessment” was started, with the main objective of coupling separate exposure and effect models into a chemical integrated exposure and effect ecosystem model for ecological risk assessment (ChimERA) in aquatic environments. In this work, the development of the dynamic fate and exposure sub-model is presented. The modelling unit, based on the dynamic water-sediment model DynA, now includes other compartments and sub-compartments, such as macrophytes, phytoplankton, particulate organic carbon (POC) and dissolve organic carbon (DOC). Moreover, the sediment compartment was divided in a number of layers to account for the spatial variability of exposure in benthic populations. After a preliminary sensitivity analysis, some simulations were performed to illustrate the effect of the most influential parameters (e.g., chemical emission, POC and DOC concentrations, macrophyte density, etc.) on exposure levels of three selected chemicals. A preliminary scenario was also prepared to include some of the parameters which change over a year cycle (water fluxes, macrophyte and phytoplankton growth, DOC/POC production, etc.). The modelling approach presented in this work, combined with the effect sub-model included in the ChimERA framework, could be a vital tool for the identification of those environmental and ecological conditions where risk is expected to be highest. *Acknowledgement* - The ChimERA project is financed by the Long-range Research Initiative of CEFIC (www.cefic-lri.org) (project code: LRI-ECO19).

270

Which exposure profile counts? Estimating worst case scenarios based on ecotoxicological relevance

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Current risk assessment procedures for chemicals in the aquatic environment consider realistic worst case scenarios determined by the highest predicted environmental concentration (PECmax) of this chemical among the calculated exposure scenarios. However, the time-dependent concentration dynamics of the estimated exposure scenarios are not considered in the selection of worst-case scenarios yet. In this study we present a generic concept to compare the ecotoxicological relevance of several different dynamic exposure scenarios and to identify the worst case scenario by applying TKTD models. The TKTD-model GUTS of fourteen different organism-substance combinations with various aquatic freshwater species and chemicals with different modes of action (including fast effects, carry over toxicity and delayed effects) were used to simulate survival rates for different exposure scenarios. In addition, TKTD parameter values were randomly generated and added to the model in order to obtain well-founded and holistically applicable results. The selected exposure profiles were a combination of calculated realistic scenarios and theoretically possible concentration changes over time. We used in total more than 400 different exposure scenarios in variable test replicates. The ecotoxicological relevance of the exposure profiles, characterized by the simulated mortality rate of the different organisms and chemicals, was converted into an ordinal scale (scenario ranking).This ranking was subsequently compared with the respective ranks of the scenarios based on different scenario descriptors (PECmax, AUC, etc.). We showed that the use of the PECmax as a driving descriptor for the impact of an exposure profile is an imprecise and often wrong method to estimate the risk of a scenario. It does not necessarily correlate with the effective impact of the scenarios towards organisms. This can lead to an underestimation of exposure profiles and to the choice of wrong worst case scenarios. However, using the descriptors PECmax and AUC in combination (min(PECmax, AUC)), by selecting the lower of both ranks for each scenario, leads to a protective prediction value for the ecotoxicological relevance of scenarios. With this descriptor the ecotoxicological impact of exposure scenarios can be compared and predicted protectively. This could be another step towards a more realistic link between exposure and effects and leads to a more thorough environmental risk assessment.

Advancements in life cycle impact assessment and footprint method development (II)

271

Combining spatially distributed land and water use impacts to an endpoint of global species extinction and application to a Kenyan case study

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Assessment of ecosystem damages made large progress during past years. However, none of the currently utilized methods on endpoint level take vulnerability of species and global extinction risk of species into account. We used recently developed methods for assessing biodiversity loss from land and water use in terms of “global species-equivalent loss” that account for these issues. Data on mammals were available for both land and water use assessments, while 3 additional taxa (birds, amphibians, reptiles) were included for water use. We discuss different weighting schemes to arrive at a common endpoint. We illustrate the application in a case study of 1 kg of sugarcane, tea and flower production in Kenya. For the land use assessment, characterization factors (CFs) were calculated

for mammals for both occupation and transformation impacts. Flower production had the highest CFs (1.1E-07 species-eq·yr/m^2 for transformation and 5.7E-10 species-eq/m^2 for occupation) because the production region is home to very vulnerable species. Final impacts on biodiversity were 6.7E-09 species-eq·yr/kg for flowers, 2.1E-10 species-eq·yr/kg for tea and 1.5E-12 species-eq·yr/kg for sugarcane. Impacts were for all crops dominated by transformation. For the water use assessment, we assumed that tea and sugarcane were irrigated with surface water, flowers were irrigated with both surface and groundwater. CFs varied between 1.5E-15 species-eq·yr/m^3 for reptiles (flowers) and 9.7E-09 species-eq·yr/m^3 for waterbirds (sugarcane). 3 weighting schemes were applied for aggregating across taxa within water use: 1) all species have equal weight, 2) weighting according to species richness of taxa, and 3) weighting according to average vulnerability of taxa. Results differed substantially for the different weighting schemes. Impacts for sugarcane were between 3.9E-10 – 1.5E-09 species-eq/kg and for flowers and tea between 1.6E-13 – 6.8 E-13 species-eq/kg and 3.1E-10 – 1E-09 species-eq/kg, respectively. For total impacts on mammals, land use impacts contributed between 14% (sugarcane) and almost 100% (flowers) to the total damage. Considering all taxa (with a weighting scheme), the share of land use impacts was between 0.1 – 0.4% (sugarcane) and 99.9 % (flowers). The importance of water use impacts increased due to more taxa and species considered. With the new unit, differences between impacts, due to different species richness and vulnerabilities were revealed.

272

The water accounting and vulnerability evaluation model (WAVE): Considering atmospheric evaporation recycling and the risk of freshwater depletion in LCA and water footprinting

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Aiming to enhance the analysis of freshwater consumption along products’ life cycles, the water accounting and vulnerability evaluation (WAVE) model is introduced. On the accounting level, the atmospheric evaporation recycling within drainage basins is considered by means of the basin internal evaporation recycling (BIER). Results show that, depending on the climatic conditions and the size of the basin, up to 38% of evapo(transpi)rated water can return to the originating basin via precipitation. When taking into account the runoff fraction, i.e. the hydrologically effective share of precipitation, 10-33% of evapo(transpi)ration return as blue water in basins in the Himalayas, Alaska, south-east Asia, and the North of South America. In order to allow for an assessment of potential consequences resulting from water consumption, the vulnerability of drainage basins to blue water depletion is evaluated. This vulnerability approach distinguishes the WAVE model from conventional characterization models describing consequences on resources. Rather than “predicting” impacts on freshwater resources, WAVE denotes the risk that water consumption in a certain region will lead to freshwater depletion. This risk of freshwater depletion (RFD) can be determined by multiplying the effective water consumption in each basin with its corresponding water depletion index (WDI). WDI denotes the vulnerability of drainage basins to freshwater depletion based on physical blue water scarcity. In contrast to previous works, water scarcity is measured based on a consumption rather than a withdrawal-to-availability ratio and accounts for the presence of ground and surface water stocks for the first time. In order to consider absolute freshwater shortage in addition to relative scarcity, WDI is automatically set to the highest value in (semi-)arid basins. WDI is at the highest level in many drainage basins located in Central Asia, India, Saudi Arabia, Australia, Northern and Southern Africa, Mexico, the south-west of the USA, and the Andes. In contrast, little or no freshwater resource depletion is caused by water consumption in most basins located in Russia, Canada, Northern Europe, or around the equator. A concept is presented, how this advanced scarcity index can be used as a starting point for assessing impacts on resources, human health, and ecosystems on the endpoint level.

273

Alternatives to GWP in LCA: temperature metrics and explicit time profiles

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The Global Warming Potential (GWP) for different time horizons (TH) was introduced by the International Panel on Climate Change (IPCC) in 1990 as an illustrative example of a potential global warming metric. Nevertheless, GWP with TH = 100 years has become the nearly exclusive emission metric used in Life-Cycle Assessment (LCA), as well as in other fields. The main criticisms of GWP regard 1) its lack of clear physical meaning in terms of temperature change, 2) the treatment of time, including the arbitrary choice of a TH, and 3) the fact that GWP is an indicator of cumulative warming which is less suitable for some climate impacts such as those associated to reaching an irreversible tipping point. For instance, because GWP assigns equal weight to emissions irrespective of the policy target and the proximity to it, it is inappropriate in the context of policies aiming at maintaining temperature changes below a given target. A few recent initiatives and research works have addressed some of these issues in the LCA community. However, a critical discussion about global warming characterization in LCA is needed since a wider range of metrics are today available for aggregating the climate impacts of different emissions to common units (e.g., kg CO_2-equivalent). Metrics based on temperature like the Global Temperature change Potential (GTP) evaluates the impact on surface temperature at a given point in time and are therefore more compatible with global policy mitigation targets. To this regard, the new 5th IPCC Assessment Report has produced characterization factors for both GWP and GTP. Here we show, using LCI data from default case studies, the large importance that the choice of a metric may have for the global warming impact category. Climate impacts based on instantaneous temperature changes differ from those based on the traditional GWP, as they are generally smaller in magnitude and shorter in time. Beside normalized indicators, the explicit inclusion of time transparently shows the time dependence of the climate effect of emissions and enhances the understanding and interpretation of the climate impact potential. The role of metrics other than GWP and their additional insights should be further investigated by LCA practitioners, especially when analyses are intended to support policy makers in the identification of the best climate change mitigation strategies.

274

Estimation of Effect Factors for application to marine eutrophication in LCIA

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Marine eutrophication is defined as the set of ecosystem responses to nutrient loadings in the photic zone of marine waters. The nutrients enrichment boosts primary production and subsequent degradation of this organic matter by heterotrophic bacteria in bottom waters results in the consumption of dissolved oxygen (DO). Impacts to ecosystems health and local economy may rise from changed communities’ composition, species interaction, decrease of water quality and depleted DO in bottom waters down to hypoxic or anoxic levels that may affect the survival of benthic species. The impacts of hypoxia on biota depend on the severity, frequency and duration of the exposure to low DO, and both acute and chronic effects can be expected. Exposure to extreme or prolonged hypoxia leads to mass mortalities, but hypoxia also induces many different sub-lethal responses in organisms at the behavioural, physiological and ecological levels. The geographical distribution of relevant benthic, demersal, or benthopelagic species (n=58), available from a dataset of sensitivities of individual species to hypoxia, was found. These were grouped into 5 climate zones - polar, subpolar, temperate, subtropical, and tropical. Species Sensitivity Distribution (SSD) curves were produced to estimate the Potentially Affected Fraction of species (PAF) at different levels of DO. For the application in Life Cycle Impact Assessment (LCIA), the distribution of the sensitivities of individual species to hypoxia is used to estimate the sensitivity to low DO levels of the communities found in each climate zone. The distribution is used to estimate the HC50, i.e. the concentration of DO (intensity of the stressor) affecting 50% of the species above their EC50 level. Characterisation Factors (CF) are used in LCIA to convert emissions and resources consumed into impact potentials for specific impact categories. The CF integrates the Fate Factor (FF), the habitat Exposure Factor (XF), and the Effect Factor (EF). The EF expresses the change of effect due to a variation of the stressor intensity and it is calculated by EF=ΔPAF/(Δ[O2])=0.5/HC50, in accordance to the average gradient method. Preliminary EF results were produced for the 5 climate zones together with a global default. The spatial differentiation obtained for the EF results was found essential to increase the discriminatory power of the model. This approach will be combined with a suitable methodology for the FF and XF.

275

From methodological developments to actual action plans in the industry: example of water availability footprinting applied to electricity production

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Over the last few years and in parallel with climate change, sustainable water use has been receiving more and more attention. In particular, water use in energy production has become an important issue. Therefore, there exists a need within utility companies to measure their water footprint and identify hot-spots where water scarcity is a potential issue and intervention may be required. The objective of this study is twofold. It aims first to illustrate how the existing water availability footprint methodologies apply to electricity production and discusses the applicability of the three methods and the potential improvements needed in an industrial context. 13 different power plants were modeled to calculate their water availability footprints according to 3 methodologies : Pfister et al., 2009, Boulay et al., 2011 and Frischknecht et al., 2006. All methods have been considered through characterization factors at the watershed level, thus implying modification of the default factors available in Quantis Suite. The model was built-up so that the water footprint can be evaluated separately for: (i) fuel supply chain, (ii) operation of PP, (iii) cooling water, (iv) water demineralization installation and (v) infrastructure building. The results obtained within this study show that, from an industrial

perspective, it remains impossible to put forward one method based on the state of the art, as the ranking amongst power plants and the relative contributions of direct and indirect operations may differ between different methods, even when differences in water consumptions are large, and hence the prioritization of action plans. This is clearly a barrier to the deployment of such methodologies by the industry, as there is a need of robustness of the methods in order that they can be used as decision making tools. This study shows that there are still some gaps to fill before a water availability footprint methodology can be recommended, at least to be applied to electricity production. The main difficulty is to choose amongst available water availability footprints, given that results may be very different and imply different improvement actions. A consensus is thus needed, either to choose one of the methods or to build another one. An analysis based on statistics could be useful to identify, for a given sector or product, the most sensitive parameters of each of the methods, and the common characteristics.

Risk assessment of chemical mixtures: strategies, bottlenecks, and the steps ahead (II)

276

Nutritional modulation of environmental pollutant toxicity: Implications for risk assessment

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An emerging body of evidence correlates persistent environmental toxicants, such as dioxins and PCB mixtures, with human diseases like diabetes and atherosclerosis. Many communities that reside in and around Superfund and related hazardous waste sites face daily contaminant exposure risks in addition to the impact of a multitude of other physiological and social stressors. One such confounding stress, sub-optimal nutrition, has now been shown to exacerbate toxicant-induced disease. Toxicants and poor nutrition both promote chronic inflammation and oxidative stress and have been shown to work synergistically to promote a more profound disease phenotype. Interestingly, new evidence now shows that diets focused on healthful nutrition (e.g., diets centered on fruits, vegetables and healthier fats) may be able to counteract the detrimental effects of persistent organic pollutants such as PCB mixtures. We have shown that mice supplemented with the tea catechin EGCG, a polyphenol, are protected against PCB-induced inflammation and oxidative stress by exhibiting a more efficient antioxidant response. Similar results have been seen in mice supplemented with DHA, a long-chain omega-3 PUFA, and subsequently exposed to coplanar PCBs. Although complete remediation of persistent environmental pollutants may be preferred to eliminate their contribution to disease pathologies, this process is extremely time-consuming and cost-prohibitive. Therefore, it is necessary to identify easily available modulators of toxicant-induced disease. Bioactive food components such as polyphenols and omega-3 polyunsaturated fatty acids may act as a sensible means to provide physiological “buffers” against toxicant-related diseases. This new paradigm that the nutritional status of an individual can impact the toxicity of pollutants has important ramifications for risk assessment and exposure sciences. Understanding the nutritional makeup and tendencies of a community will allow scientists to better measure and estimate true risk of a specific pollutant or mixture. As part of the University of Kentucky’s Superfund Research Center, active community engagement aims to educate and inform communities directly impacted by daily exposures to persistent environmental pollutants.Through nutritional education, counseling, and demonstrations we empower individuals to make smarter eating decisions that can help to buffer against chronic exposures to environmental contaminants.

277

EU wide campaign exercise on bioassays and chemical mixture effects

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Thousands different chemicals are discharged into the environment from agriculture, industry, medical facilities, house-holds. Currently, there is an increasing concern for the environmental impact of mixture of compounds since the additive and eventual synergistic effects are unknown and could produce serious adverse effects. Recently, a document from the European Commission on combination effects of chemicals highlighted the need to ensure that risks associated with chemical mixtures are properly understood and assessed. To address this issue, a joint-effort of 16 European and associated research groups participated to an exercise to test a synthetic reference chemical mixture on the own routine bioassays to investigate the chemical mixtures effects. The reference material included class of pesticides, pharmaceuticals, industrial products, heavy metals and polyaromatic hydrocarbons. The mixures were prepared, each compound at Equivalent Quality Standard (EQS) value, the safety limit concentration allowed by the European Water Framework Directive, (WFD).The bioassays proposed by the groups could cover the entire ecosystem from bacteria to fish as well *in vitro* assays providing an unique scenario from ecological risk assessment perspective. The results showed that effects were observed at very low concentration on algal-bacteria composition in a marine microcosm, immobilization in crustacean, fish embryo toxicity and frog embryo development. We conclude that some precaution on the chemical mixture assessment should be taken even in case the individual compounds are present at EQS, the safety limit concentration under European legislation.

278

Ecotoxicological assessment of immersion samples from façade render

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To protect façade renders from growth of bacteria, fungi and algae, biocides can be added to a render before it is applied onto a façade. A comprehensive protection can be achieved by combining several biocides. During rain events and over time, biocides will gradually leach out and thus have the potential to affect soil or aquatic ecosystems. In this study the leaching behaviour of three biocides (terbutryn, OIT, DCOIT) from three render formulations was evaluated: one render contained free biocides, another encapsulated biocides and a control render had no biocides. The renders were applied onto extruded polystyrene panels and water samples were generated over 9 immersion cycles of the panels in accordance with standard EN 16105. Concentrations of the biocides were measured using LC-MS. The toxicity of the first and ninth immersion samples was determined using bioassays. Toxicity to aquatic organisms was evaluated by assessing inhibition of photosynthesis and algal growth rate, inhibition of bacterial luminescence and inhibition of daphnid population growth. Toxicity to soil organism was assessed by determining avoidance behaviour of worms and reproductive output in springtails. Encapsulation reduced the leaching of terbutryn, OIT, and DCOIT 4-, 17-, and 25-fold compared to free biocides. Generally, the toxicity of water from render containing encapsulated biocides was always lower than that of render with free biocides and toxicity was 4- to 5-fold lower for the ninth immersion day compared to the first immersion day sample for both free and encapsulated samples. For the aquatic organisms, inhibition of photosynthesis was the most sensitive endpoint, followed by algal growth rate, bacterial bioluminescence and daphnid reproduction.

At all tested sample concentrations, none of the samples with biocides caused effects on soil organisms. Results from bioassays matched quite well with expected bioassay responses based on chemical analysis and the toxicity of the individual biocides. It could be concluded that the toxicity of given concentrations on algae is explained by terbutryn whereas the toxicity on bacteria and daphnids is caused by DCOIT and OIT. The results thus indicated that other components in the render did not add to the toxicity of the individual biocides. Overall, the approach combining a standard leaching test with standard bioassays is very promising to evaluate the ecotoxicity of biocides that leach from façade renders.

279

Qualitative environmental risk assessment of photolytic transformation products of Iodinated X-ray contrast media (ICM) Diatrizoic acid

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One of the worldwide used genres of chemical for intravascular administration is ICM which heavily contributes to the total adsorbable organic halides load in municipal and hospital effluents. Therefore it is necessary to assess the environmental risk caused by these ICMs. In this study, a combination of analytical methods, *in silico* predictions, biodegradation testing and *in vitro* toxicity screening using luminescent bacteria (modified luminescent bacteria test [LBT]) for initial analyses of the environmental fate and effects imposed by the photodegradation TPs (PTPs) of Diatrizoic acid (DIAT). DIAT was photolytic transformed under UV irradiation. Its PTPs and a degradation pathway were elucidated. DIAT and PTPs were assessed by the USEPA PBT profiler which indicated DIAT as persistent chemical and its photo-TPs as comparatively less persistent and possess no bioaccumulation threat to aquatic organisms. The mixture of PTPs was subjected to standardized OECD 301 biodegradation test assays. The results indicated that a few of the PTPs were comparatively better biodegradable than DIAT itself confirming the *in silico* readily biodegradable predictions of PTPs. These comparatively better biodegradable PTPs were identified. Although, the slight inhibitory effect was observed against the inoculum’s bacteria which indicates that some of the formed PTPs might be toxic to bacteria. However, few PTPs were predicted by *in silico* models to be toxic against environmental bacteria. These predicted toxic PTPs were also identified to be non-biodegradable. LBT assays demonstrated an increase of acute and chronic toxicity against *Vibrio fischeri* for the mixture of PTPs with highest inhibition after 32 and 64 min irradiation. Although these predicted toxic PTPs were formed in low yield but might have additive or synergistic mixture effects in LBT and the inhibition in biodegradation test assays. Furthermore, few photo-TPs were predicted to be active for mutagenicity and genotoxicity which indicates the need for further testing to confirm these predictions. *In silico* predictions and *in vitro* test results were in direct correlation but the formation of free Iodine during photodegradation might be responsible for the inhibition against bacteria in LBT assay which require further testing to confirm. However, the elucidations of the structural formula of TPs are sufficient to collect appropriate data as a basis for an environmental risk analysis.

280

Bioanalytical and Chemical Evaluation of Swimming Pool Water

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The use of chemical disinfectants in swimming pools is vital to prevent pathogens from causing adverse human health effects. However, toxic disinfection by-products (DBPs) are formed from the reaction between disinfectants and organic/inorganic precursors. The aim of this study was to assess the toxicity and presence of DBPs in various swimming pool types in Brisbane, Australia. The combination of chemical and bioanalytical tools was applied to evaluate the toxicological relevance of the known fraction of DBPs compared to the unknown fraction of total contaminants. Further, we assessed one pool fortnightly during 6 months after a complete water exchange. The time series revealed that chlorinated organic compounds were higher concentrated compared to tap water while the brominated compounds decreased over time, indicating a removal and/or volatilisation without reformation. Haloacetic acids (HAAs) were the dominating DBPs with highest concentrations for dichloroacetic acid and trichloroacetic acid (up to 26× higher than the Australian drinking water guideline value of 100 µg/L). Up to 100% of the organic halides (AOX) could be explained by the detected HAAs, which is extraordinary high compared to DBPs in drinking where the knowns often explain less than 50%. Despite the high agreement between AOX and HAAs, HAAs explained only up to 4% of non-specific toxicity (Microtox) and ≤1% of oxidative stress response (AREc32) and genotoxicity (UmuC). This indicated the presence of other contaminants, which contributed to the overall toxicity in the pool water samples. The p53 induction was masked by cytotoxic effects for most of the samples and is hence not further discussed. The bioassay response of pool water was within the range of tap water indicating a comparable level of toxicity. While chlorinated organic compounds are higher concentrated in pool water, the toxicologically more relevant brominated organics decreased over time and were generally considerably lower compared to tap water. Despite their predominating concentrations, HAAs account for < 5% of the bioassay responses, indicating a) a low toxicological relevance of both HAAs in the comlex mixture of the samples and b) the presence of other contaminants, which contributed to the total toxicity. This study demonstrates the necessity of combining chemical and bioanalytical evaluation to provide a comprehensive picture of real-world mixtures for water quality and risk assessment.

Chemical pollution in sustainable management of aquatic ecosystems – challenges and approaches from a Swiss perspective (II)

281

The Swiss water policy for tackling chemical pollution

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Chemicals, often referred as micropollutants, such as pharmaceuticals, personal care products, pesticides, biocides, or endocrine disruptors, are organic trace contaminants present at very low concentrations in the water bodies (at nonagrams or micrograms per liter). Most of these substances are contained in municipal wastewater and discharged into receiving waters, since not sufficiently eliminated by state-of-the-art municipal wastewater treatment plants. Especially, rivers with inadequate dilution of treated wastewater inflows are subject to elevated micropollutant concentrations. These findings suggest that several measures have to be taken in order to further improve water quality: For example, the International Commission for the Protection of the Rhine (ICPR) stated in a recent strategy paper (Report No. 203) that emission into water bodies should be reduced. Moreover, several types of actions are discussed, which are: measures at the source (e.g. adaptation of product registration, optimization of production processes, regulation of use and disposal), decentralized measures, as well as centralized measures in municipal wastewater treatment plants (e.g. implementation of further treatment steps such as ozonation or the application of activated carbon). In Switzerland, 100 out of over 700 municipal wastewater treatment plants will be upgraded with advanced treatment processes in a targeted approach within the next 25 years. In that way, the total micropollutant load in municipal waste water will be reduced by 50 per cent, at estimated total investment costs of about CHF 1.2 billion, which is considered a justifiable cost/benefit ratio. In parallel, further steps will be evaluated, such as measures at the source or reducing diffuse inputs. Latter is currently addressed by several studies providing an overview of current measures, focusing amongst others on the field of agriculture (e.g. development of a national action plan) or urban water drainage.

282

Bank infiltration, river restoration and effects on drinking water: the relevance for Switzerland

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Around 80 % of Swiss drinking water is obtained from groundwater. 25 to 30 % of this groundwater is abstracted from aquifers that are hydraulically directly connected to a surface water body, mostly rivers. Here, a large portion of the extracted groundwater is freshly infiltrated surface water. During the percolation of the surface water through the aquifer an improvement of water quality is achieved by a series of physical, chemical, and biological processes (e.g. adsorption, reduction, biodegradation). With this, bank filtration can be seen as pre-treatment measure in the overall treatment chain. Consequently, Swiss regulations defined that the travel time for the subsurface passage from the surface water body to a drinking water well has to be at least 10 days to ensure that most microbial pathogens are filtered out or die off before they can reach the well. This regulation is also basis for the prohibition of river restoration measures next to bank filtration sites. River restoration in general increases river dynamics, environmental heterogeneity, biodiversity, and also the exchange of surface water and groundwater. Swiss legislation requires river restoration actions also as part of flood protection measures. This and the improvement of the ecomorphological status of selected river sections, mainly located in the Swiss plateau, are the main reasons for the decision of the Swiss government to restore river sections of a total length of about 4´000 km in the upcoming decades. These measures will have an impact on the river-river corridor-aquifer system and with this, also on bank filtration sites. To provide clean drinking water also in the future, the impact of numerous restored river sections on the groundwater needs to be investigated. With this an adequate understanding of the underlying hydrogeological and ecological processes can be developed.

283

Artificial groundwater recharge: conflicts between river water quality and drinking water needs?

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Naturally occurring recharge of groundwater is a well know process in humid areas. In order to enhance the amount of stored and available groundwater, water may additionally be recharged. Artificial groundwater recharge is a technical measure to increase available groundwater resources while using the natural attenuation capacity of the subsurface passage to improve the water quality. Use of artificial groundwater recharge has rapidly increased in Europe, USA, and in Australia in recent years not only for drinking water use but also as an efficient means of recycling stormwater or treated sewage effluent for non-potable and indirect potable reuse in urban and rural areas. During artificial groundwater recharge, surface water (e.g. from infiltration ponds) or treated sewage water is infiltrated into the aquifer and is extracted after subsurface passage at extraction wells. Artificial groundwater recharge offers opportunities for producing safe drinking water supplies where they do not exist. However, it relies first and foremost on the presence of suitable aquifers and safe sources of infiltration water together with effective water policies. Recently, a major concern in the application of artificial groundwater recharge for drinking water abstraction is the occurrence of persistent organic and inorganic compounds even at trace levels and pathogenic microorganisms. Therefore, research has focused on the removal of these contaminants including pharmaceutically active compounds like primidone, carbamazepine, and sulfamethoxazole and on removal of pathogens. In many places, not only groundwater is limited but also surface water. Artificial recharge of groundwater with river water may limit the amount of river water available for the river ecosystem, for downstream users, for industrial use of surface water including hydropower and for recreational purposes. The quality of the infiltrating water determines the overall quality of groundwater in the long-term which leads to the demand of infiltrating water with best quality only if drinking water is to be produced. Finally, due to climatic change these systems may have to be reevaluated based on the local impacts of climate change.

284

The Swiss strategy abating micropollutants: What can be learned for outside Switzerland?

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Evolutionary, multigenerational and epigenetic effects of pollutants: providing scientific support to long-term ERA

285

Cd-resistance in field Gammarus populations: genetic adaptation, transgenerational effect, or selection of phylogenetic cryptic lineages?

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We have recently identified a field population of the crustacean *Gammarus fossarum* historically-exposed (geochemical background) to cadmium (Cd) that exhibits a resistance transmissible to offspring. This population constitutes a good case study to investigate mechanisms underlying population's resistance to long-term exposure to contamination that are of deep concern for ERA, especially in the field. In order to gain insights into the determinism of the Cd-resistance observed in this historically-exposed population, we applied quantitative genetic protocols both in two Cd-naive populations and in the resistant one. In addition, because *G. fossarum* is known to constitute a complex of cryptic species with potentially heterogeneous sensitivities to contaminants, we also tested whether this resistance could be explained by the selection of a peculiar phylogenetic group among the *Gammarus* diversity (based on COI genotyping). Our work reveals that the Cd-resistant population does not belong to a specific lineage and sustains that its resistance to Cd is due to transgenerational maternal effect induced by exposure in the field. Whether this transmissible resistance is fixed (phenotypic plasticity) or transitory (flexibility) is still to be investigated to decipher the role of acclimation in population resistance. Also, parental effects seem to be a key endpoint to understand the modification of sensitivities to contaminants in the field, and taking into account this phenomenon is mandatory to improve the relevance of ERA.

286

Molecular mechanisms behind snail metal sensitivity: Cadmium metallothionein gene transcription involves activation of metal responsive elements

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Many animal species are able to cope with elevated Cd concentrations in their environment by upregulation of Metallothionein (MT) genes and binding the metal to the expressed MT protein [1]. In vertebrates and other animal species, MT gene transcription is mediated through the binding of the Metal Responsive Transcription Factor 1 (MTF-1) to Metal Responsive Elements (MREs) in the promoter region of the MT gene. Only little information was so far available about Cd-dependent MT transcription activation in terrestrial gastropods. Although their CdMT gene contains putative MREs, they apparently lack the MTF-1 [2], which raises the question of about how they upregulate their CdMT gene under metal exposure. In the present study, Roman snails (*Helix pomatia*) were exposed to elevated Cd concentrations over a period of 14 days, in order to track the accumulation of Cd in the midgut gland and to quantify, concomitantly, the transcription of the *CdMT* gene, as well as the expression of CdMT at the protein level. Low MT transcript copy numbers, low Cd concentrations and accordingly depressed CdMT concentrations were detected throughout the whole experiment in control animals. In contrast to this, Cd-exposed individuals showed increasing Cd concentrations within the midgut gland, along with a transient MT mRNA transcription peak on day 5 of exposure, whereas the respective MT protein concentrations increased from the beginning of the experiment reaching a persistent elevated level until the end of Cd exposure. In order to verify whether the MREs identified in the promoter region of the snail *CdMT* gene might be involved in its Cd-dependent transcriptional activation, DNase I footprinting was applied to control and Cd-exposed individuals. It appeared that the MRE b/c elements in the proximal promoter region of the Roman snail *CdMT* gene are functional as binding sites for transcription factors. Since snails do not possess an MTF-1, the nature of Cd-dependent transcription factors in their *CdMT* gene remains still unknown, suggesting the evolution of a detoxification mechanism in terrestrial gastropods that differs from most other animal species.

287

Cu and Zn selection leads to adaptation and heterozygote excess in a natural Daphnia magna population without affecting tolerance to novel stressors

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Natural populations can genetically adapt to chemical stress through the process of micro-evolution. As a consequence the allelic constitution of the population is altered and overall population genetic diversity is reduced. We exposed a natural *Daphnia magna* population to a control, a Cu (180μg Cu/L), and a Zn concentration (760μg Zn/L) for 10 weeks under semi-field conditions to assess whether adaptation could indeed occur on such a short time scale. Populations which evolved under Cu and Zn during the microevolution experiment produced significantly more offspring under Cu and Zn exposure than the original population. At the end of the micro-evolution experiment DNA samples were taken fom the original population, as well as from the evolved aquaria populations and microsatellite genotyping was performed using a total of 13 microsatellite markers. We estimated the inbreeding coefficient (*F*), which is a measure of homozygous vs. heterozygous excess, where negative values specify an excess of heterozygotes, while positive values indicate an excess of homozygotes and we observed negative inbreeding coefficients (excess of heterozygotes) in the Cu and Zn adapted populations, while inbreeding coefficients were positive (excess of homozygotes) in the original and control evolved populations. After confirming adaptation to Cu and Zn, we assessed the cost of adaptation, whereby populations evolved under and adapted to either Cu or Zn, may become less tolerant to novel stressors. We compared the tolerance of the metal adapted populations to the original and control evovled populations to temperature and cyanobacteria diet stress (*Microcystis aeruginosa*). We did not detect noticable differences in tolerance to new stressors. We did notice that survival time of the population evolved at 760μg Zn/L was significantly lower relative to the control evolved population at 28°C. Overall our results suggest that adaptation to Cu and Zn leads to an excess of heterozygotes but that such an adaptation comes neither with a cost of tolerance nor a distinctive advantage when the adapted populations are faced with novel stressors.

288

Does the nutritional status of parental Chironomus tepperi midges influence the sensitivity of its offspring?

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Environmental stimuli can induce plastic variations in the life history traits of an organism without corresponding alterations in the genome.Such variations can be transmitted to the offspring through the inheritance of factors other than alterations in the DNA sequences (non-genetic inheritance or transgenerational effects).

Nutrition is an environmental factor known to cause generational effects also in humans. The present study considers the effects of parental nutritional status in *Chironomus tepperi* on some life history traits and metal sensitivity of offspring. This study is divided in two parts:\n The first experiment was designed to verify whether transgenerational effects are inducible in *Chironomus tepperi* by varying the nutritional status of the parents. This was achieved rearing the parents in three different feeding regimes (high, standard and low) and the offspring in the same regime (standard). The parental feeding regime "low" was chosen with the purpose of causing stress conditions. The second experiment aimed to evaluate whether the nutritional status of parental *Chironomus tepperi* influenced the sensitivity of offspring to zinc. We tested whether exposing the offspring of stressed parents to a stressor different from that experienced by the parents will reveal an advantage. Therefore the offspring was exposed to a sublethal zinc concentration (0.02 mg/L). Nutrition triggered transgenerational responses in *Chironomus tepperi*. Offspring of starved parents had a shorter larval development time in all treatments (in average 2 days shorter) and lower sensitivity to zinc. The opposite trend was observed in fecundity: offspring of well-fed parents appeared to benefit from the nutritional status of their parents, and produced bigger egg-ropes.
Transgenerational effects triggered by nutrition or nutritional stress are common in *Chironomus tepperi* and potentially in other *Chironomus* species. This can have major implications in ecotoxicology. Depending on the environmental conditions experienced by the parents, offspring may exhibit phenotypic variability and fitness differences. Species sensitivity may vary across populations and over time. This could be the cause of inconsistencies and variability among laboratories or within the same laboratory.

289

Effects of chronic gamma irradiation: a multigenerational study using Caenorhabditis elegans

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The assessment of environmental impact of exposure to ionizing radiation (natural and ubiquitous phenomenon enriched by human activities) has become a major concern. However, this environmental risk assessment is currently hampered by the lack of knowledge, and hence, is often based on extrapolation from data obtained for acute exposure. Studies on chronic exposure over several generations are so needed to understand the disturbances related to ionizing radiation and their possible consequences on the population. Regarding this background, we assessed the effects of chronic exposure to ionizing radiation over three generations of the ubiquitous nematode *Caenorhabditis elegans*. In this study *C. elegans* were chronically and individually exposed to gamma radiation (dose rates ranging from 6.6 to 42.7 mGy/h). The evolution of growth and reproduction (here, cumulated number of larvae per individual) of individuals were followed daily. Comparisons within and between the generations of *C. elegans* subjected to different exposure statuses: (i) three generations continuously exposed (F0, F1, and F2) and (ii) parental generation exposed (F0) and the following generations placed in recovery (F1' and F2') were performed. Our experiment showed no significant difference in growth between the control and the exposed individuals whatever the generation and the exposed status. However we observed a decrease in the reproductive ability between F0 and F2 at the highest dose rate (42.7 mGy/h). We also observed significant differences in the same generation subjected to different exposure statuses (exposed (F1) or recovery (F1')). Surprisingly, the non-exposed generation (F1') laid out less number of eggs than the exposed generation (F1). Our results confirmed that reproduction is the most sensitive endpoint affected by ionizing radiation and revealed transgenerational effects from parental exposure in the second generation (F1') and the third generation (F2). Using these results on reproduction, molecular and cellular effects of chronic exposure to ionizing radiations on germline are examined to better understand the mechanisms underlying the observed effects

Fish model species in environmental toxicology (III)

290

Novel approaches to assess the effects of glucocorticoids in zebrafish embryos

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Synthetic glucocorticoids (GCs) are widely used in medicine. These compounds have been detected in the aquatic environment in the ng/L range. GCs mimic cortisol, the natural stress hormone, by acting through the glucocorticoid receptor (GR) and altering related gene expression patterns. Potential adverse health effects of GCs may range from teratogenesis to impairment of stress response, glucose metabolism and immunosuppression. In our work, we use zebrafish embryos to investigate if exposure to a relevant model GC, clobetasol propionate (CP), would result in the aforementioned adverse effects. The respiratory burst assay (RBA) was used to examine whether GCs can have an effect on macrophage functions, namely, their ability to produce reactive oxygen species (ROS) in response to infectious agents. Embryos exposed to 1000 nM CP from 1 to 120 hours post fertilization (hpf) showed significantly reduced amount of ROS produced by macrophages. The outcome of these experiments indicates that GCs are able to cause suppression of the innate immune system in zebrafish embryos. Targeted proteomics analyses were conducted on tryptic peptide digests from 30-40 embryos exposed to 10 or 100 nM CP from 96-120 hpf. The proteotypic peptide targets were monitored using a mass-spectrometry based targeted proteomics technique, selected reaction monitoring (SRM). GR target genes already examined in zebrafish at the mRNA level were selected to monitor their protein products. The targeted proteins have different physiological roles, e. g. immune response, cardiogenesis, myogenesis and osmoregulation. In a pilot experiment, two protein targets, Smyhc1 and Atp2a2a showed compelling changes in expression in exposed samples, thus they are potential protein biomarkers of GC exposure. The described work supports the fact that GCs can have immunosuppressive effects in fish. So far, the initial targeted proteomics analyses revealed two potential protein biomarkers of GC exposure that may be used to develop a sensitive bioassay in order to detect and identify unknown GR-active compounds with effect-directed analysis (EDA) in environmental water samples. Further experiments examining other potential biomarkers are ongoing.

Keywords: zebrafish embryo model, environmental glucocorticoids, immune response, protein biomarkers

291

Toxicity of the mycotoxin zearalenone in fish

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The world-wide occurrence of mycotoxins including zearalenone (ZEN) in grains and cereals is a major problem leading to contamination of feed for farm animals and fish. Mycotoxins in feed have been shown to posess multifold detrimental effects on fish. Moreover, contamination of fish feed with mycotoxins has been found to occur. Consequently, mycotoxins have become a worldwide interest. Especially ZEN has been shown to act as a typical estrogenic compound showing high estrogenic potencies *via* estrogen receptors of rainbow trout. In fish, the mechanisms of toxicity of ZEN have not yet been demonstrated but effects on reproduction and development have been described. In mammalian systems it has been concluded that it is unlikely that the toxicity of this substance is solely due to its endocrine potential because other mechanisms, such as oxidative stress, could be involved. Generation of oxidative stress is involved in important physiological functions of all aerobic organisms. But it can also lead to disturbances of metabolic pathways, depletions of cellular antioxidants, and damage to macromolecules, including DNA, proteins and lipids. Thus, possible oxidative stress due to exposure to ZEN was analysed in the present study using fish cell lines to show the involvement of this pathway in the toxicity of this mycotoxin even outside mammalian cell systems. *In vivo* experiments were conducted to evaluate the relevance of the findings in whole fish.

292

Mechanisms of Selenomethionine and Hypersaline Induced Embryotoxicity in the Euryhaline Fish, Japanese Medaka (Oryzias latipes)

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As climate change worsens, the salinity of important spawning grounds in certain water-restrained estuaries, such as the San Francisco Bay, is increasing. Hypersalinity may not have direct lethal effects on adult fish, but osmotic stress may alter detoxification strategies of developing organisms. Although Selenium is an essential micronutrient, it has demonstrated embryo toxicity to fish at high concentrations and occurs in areas impacted by hypersaline conditions. Selenomethionine has been previously shown to cause oxidative stress in Japanese medaka embryos and when combined with hypersalinity decreased embryo hatch. The objective of this study was to examine potential mechanisms for embryo lethality caused by combined stressors in developing euryhaline fish. Because of the difficulties of working with fish from the Delta, Japanese medaka are a good model organism for studying the effects of multiple stressors. Japanese medaka embryos were treated with 50μM selenomethionine alone and with 12ppth salinity for 12 hours then apical endpoints and endpoints for oxidative stress, apoptosis and the unfolded protein response (UPR) were taken. Deformities were increased in selenomethionine treated embryos. However, no increase in lipid peroxidation was observed. Q-PCR was used for measurements of *CASP3A* and *BAX* transcripts. In selenomethionine only treatments expression of apoptotic transcript, *CASP3A*, was up-regulated significantly, and there was a trend towards an increase in *BAX*. Additionally, transcripts for genes regulating the UPR, *BiP*, *IRE1*, *ATF6, ATF4* and *Nrf2*, were measured. Selenomethionine significantly increased *BiP, ATF4*, and *ATF6*. Effects of salinity stress remain unclear. These results indicate embryo lethality may occur through apoptosis and the unfolded protein response.

293

FISH EARLY LIFE STAGES ASSAYS WITH SEDIMENT CONTACT

EXPOSURE AS A TOOL FOR HYDROPHOBIC COMPOUND TOXICITY ASSESSMENT

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Japanese medaka *Oryzias latipes* embryos were incubated onto artificial reference sediment during their whole development until hatching. This sediment was spiked with different environmental concentrations of three PAH complex mixtures extracted from a PAH-contaminated sediment (Seine estuary, France) and two oils (*Arabian Light* and *Erika*). Several endpoints were examined at different developmental stages including mortality and hatching success, morphological abnormalities, larvae locomotion and genotoxic effects (micronucleus and comet assay). We also examined PAH bioavailability, accumulation in tissues and impact on gene expressions by RT-qPCR. Environmental concentrations of the three PAH fractions delayed hatching, induced developmental abnormalities and DNA damage and disrupted larvae swimming activity. Accumulation of PAH was detected in embryos tissues exposed to the *Erika* fraction. *Cyp1A* transcription level was positively correlated to PAH concentration in water and embryo tissues. Significant gene transcription deregulation was observed for several genes involved in cell cycle control, DNA repair (*p53*), vitamin A (*rarα1*) and oestrogen (*cyp19b*) metabolism, respiratory chain functioning (*coxI*) and anti-oxidant defense (*sod Cu*). These changes in gene expression provided clues to better understand mechanisms of PAH mixtures toxicity in fish early life stages. This was not supported by the toxic equivalency approach that appeared not suitable for the toxicity assessment of the three PAH fractions to fish embryos and larvae. The *Arabian Light* and *Erika* petrogenic fractions, with high proportions in alkylated PAHs and low molecular weight PAHs, were more toxic to Japanese medaka early life stages than the pyrolytic fraction. These results were consistent with toxicity observed with the same PAH fraction samples in rainbow trout early life stages. Altogether, these results demonstrate the suitability of the sediment-contact assay with fish embryos for toxicity assessment of hydrophobic pollutants.

294

Motoneuron and neuromast damage as new endpoints to explain metal toxicity in the zebrafish embryo toxicity test (zFET)

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Metal exposure is still a global environmental problem and can lead to severe damages and development impairments through toxic mechanisms in organisms like fish. Many of these mechanisms of metal toxicity are still not clarified in detail. We are using the zebrafish toxicity embryo test (zFET), which is increasingly being used as a replacement test method to elucidate the potential sites of metal impact. New endpoints extend the scope of the existing assay and allow specifying the toxic action of a chemical. Therefore, metals were tested including new analysis methods for neuromast and motoneuron damage. The lateral line with the neuromasts is directly exposed to the aqueous surroundings, and hair cell excitation is triggered by water movements, influencing rheotaxis and coordination. Additionally, the hair cells of the neuromasts are homologue to the hair cells in the chochlea of mammals. This makes a neuromast damage assay with zebrafish also interesting for hearing research and the screening of ototoxic substances. In this study, wild-type embryos were exposed to the metals copper ($CuSO_4$), cadmium ($CdCl_2$) and cobalt ($CoSO_4$) for 72 hours. The embryo toxicity tests were conducted according to guideline recommendations and morphological endpoints were assessed as described by Braunbeck et al. 2006. Neurotoxic effects were investigated by immunofluorescence stainings of primary motoneurons (PMNs) and secondary motoneurons (SMNs). To quantitatively evaluate the development and damages of neuromasts, an in vivo staining method was applied using the vital dye DASPEI. Additionally, the consequences of metal toxicity and damage were assessed functional by testing the fish behaviour. Hence, a behaviour assay was performed, which analyses the escape reflex after tactile stimulation. The stained neuromasts of the 72 hpf embryos showed a concentration-dependent decrease in fluorescence for all three metals even in the lowest concentrations, 2 mg/l $CdCl_2$ and 0.025 mg/l $CuSO_4$ and $CoSO_4$. For the secondary motoneurons a slight effect increase was noticeable. The results demonstrate that the neuromast cells are generally more sensitive to metal exposure than morphological parameters, the response to tactile stimulation and the motoneuron damage. Therefore, the assessment of neuromast damage promises a valuable new endpoint for metal toxicity in the zFET.

Ecological Consequences of Exposure to Pharmaceuticals: From the Laboratory to the Field (III)

295

Does exposure to environmentally relevant concentrations of an antidepressant affect risk sensitivity of wild birds?

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Many species of birds forage on sewage treatment works and on fields fertilised with biosolids, attracted by the abundance of invertebrate prey. Many pharmaceuticals can accumulate in such invertebrates. Fluoxetine (FLU) is an antidepressant commonly prescribed to treat anxiety and depression and has been detected in a range of environmental matrices and also soil invertebrates. In order to assess whether behavioural and physiological effects result from foraging on pharmaceutical contaminated prey, we administered wild-caught starlings Sturnus vulgaris with fluoxetine-injected invertebrates over 16 weeks in captivity. Assayed over two days in isolation, anxiety related behaviours (exploration in a novel environment and responses to human disturbance) and activity levels were unaffected by chronic exposure to environmentally relevant concentrations of fluoxetine. Fluoxetine did affect physiological stress responses: As found other species, controls showed a highly variable corticosterone response on day 1, i.e. immediately following the stress of being put into isolation. This was followed by a significant reduction in stress hormone variability on day 2, characteristic of habituation by all birds to the new environment. In contrast, the variance in mean corticosterone levels of the FLU-group did not change from day 1 to day 2. Similarly, controls demonstrated the expected relationship between stress hormones and mass change: the birds showing high corticosterone on day 1 lost more body mass than controls with lower physiological responses to the stress of isolation. However, for FLU- birds, corticosterone did not predict mass change in isolation. Finally, in their home aviaries controls, as expected, fed more at the beginning and end of the day. So as seen in other avian species during winter, controls were strategically trading-off the risk of starvation against the ability to escape predation depending on the time of day. The FLU-group, in contrast, did not change their feeding rate over the day, thus their foraging behaviour showed them to be less risk sensitive to starvation and predation. Our data suggest that exposure to fluoxetine disrupts the relationships between Corticosterone, feeding behaviour and body mass regulation in birds, thus altering risk sensitivity. Further work is needed to determine whether our experimental results could decrease individual fitness resulting in changes to population dynamics.

296

Tissue-Specific Concentrations of Polar Pharmaceuticals and Personal Care Products (PPCPs) in Wild Fish from Wastewater Discharge Area

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Pharmaceuticals and personal care products (PPCPs) are considered as "pseudo-persistent" contaminants, because of their continuous loading to aquatic environments through the effluent discharge of wastewater treatment plant (WWTP). As a result, exposures and adverse effects of PPCPs on fish are becoming problems of deep concern. In our previous study, a sensitive and accurate isotope dilution method was developed for the simultaneous determination of 19 polar PPCP residues (log K_{ow} = 1.40–5.74; pK_a >3.10; pK_b >4.15) in various biological matrices (e.g., liver, kidney, and brain). The present study elucidated residue levels and bioaccumulation patterns of 19 polar PPCP in the blood, gill, liver, kidney, muscle, and brain of wild fish from wastewater discharge area. Thirteen of 19 target compounds were detected in fish tissues analyzed. Overall, higher concentrations of psychotropic agents were found in brain, liver, and kidney rather than plasma and muscle. Relatively higher concentrations of antibacterial agents were found in liver and gill. In contrast, tissue levels of analgesic agents were comparable with plasma concentrations. Among target compounds found in ambient water, antihyperlipidemic agents, ethenzamide, and warfarin with lower lipophilicity were not detected in any fish tissue analyzed. These compounds may be rapidly excreted, and consequently present at low concentrations in fish tissues. However, high bioaccumulation factors (concentration ratios: fish tissues / ambient water) of diphenhydramine in liver and kidney were found in spite of their relatively low lipophilicity. This result implies that other mechanisms such as differences in biotransformation rates by fish might be involved in the accumulation of polar PPCPs. Moreover, comparing measured fish plasma levels to available human therapeutic plasma levels, it can be suggested that haloperidol (antipsychotic agent) and diphenhydramine (antihistamine agent) impose relatively high risk. The mean brain / plasma concentration ratios of psychotropic agents ranged from 8.4 (carbamazepine) to 12 (diphenhydramine), indicating high transport of these compounds to the brain of fish as it is in human beings.

297

Occurrence of endocrine disruptors and pharmaceutical compounds and bioaccumulation in biofilm, macroinvertebrates, and fish in Mediterranean rivers

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Pharmaceuticals (PhACs) and endocrine disruptors and related compounds (EDCs) are continuously introduced into surface waters, thus, the likelihood of bioaccumulation of these compounds in exposed aquatic organisms is an ever-increasing issue of concern. The situation is of special concern in Mediterranean regions, where the WWTP effluents may represent a high percentage of some streams flow, particularly under water scarcity. This work describes the presence and bioaccumulation of PhACs and EDCs in aquatic organisms from different trophic levels – biofilm, macroinvertebrates, and fish – collected in four Mediterranean rivers inSpain. Sampling was performed in 5 points in each of four rivers to achieve a pollution gradient. A total of 20 sites were sampled during the summer of 2010 for the analysis of up to 43 multi-class pharmaceuticals and 21 EDCs in aquatic organisms from different trophic levels: biofilm, insect larvae, mussels and fish. Twelve EDCs, out of 18 detected in water and sediment, were also found in biota samples. Bisphenol A, propylparaben, TCEP and triclosan were detected in all the biofilm samples analyzed. TBEP was detected in all the macroinvertebrate samples, and in 95% of the fish samples. These compounds were also some of the most prevalent compounds in water and sediment (detected in more than 70 % samples). Compounds like caffeine and tolyltriazole, although persistently detected in water at considerable concentration in the majority of the sampling points (up to 572.2 ng L^{-1} and 874.9 ng L^{-1}, respectively), were only detected in one biota sample at very low concentration. Twenty PhACs, out of 77 found in water and sediment, were also detected in aquatic organisms. Most prevalent compound in biofilm was the antibiotic azythromycin, found in 44 % of the samples. In contrast, the anti-inflammatory drug diclofenac was the most ubiquitous compound in fish, as it was detected in different fish species and all the river basins. Compounds frequently detected in water, such as acetaminophen and ibuprofen, with concentrations around 150 ng L^{-1} in some sampling sites, were not detected in any aquatic organism.

298

Analyses of pharmaceuticals in fish collected from a subset of urban streams in the U.S. in the U.S. EPA's National Rivers and Streams Assessments

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U.S. EPA' conducted the first statistically based survey of persistent and bio-accumulative contaminants in fish from U.S. Rivers. This national fish survey was conducted under the framework of EPA'sNationalRiversand Streams Assessment (NRSA), a probability-based survey designed to assess the condition of the Nation's streams and rivers. In 2008 and 2009, field teams applied consistent methods nationwide to collect samples of fish commonly consumed by humans at 542 randomly selected river locations (≥ 5th order based on 1:100,000-scale Strahler order) in the lower 48 states during June through October, 2008-2009. Fish were collected at every sampling location and prepared as composite samples, which consisted of five similarly sized adult fish of the same species. Largemouth and smallmouth bass were the primary species collected for the study, accounting for 34% and 24% of all fish composites, respectively. The selected streams were further stratified into 160 urban streams, where a water sample was collected for pharmaceutical analyses in addition to fish. Fifty four pharmaceuticals and 6 metabolites measured in the water samples were used to prioritize 25 sites for fish tissue analyses. Criteria used for selecting sites for pharmaceutical analyses of fish fillets included: nearness to a wastewater treatment plant discharge, broad continental U.S. distribution, low flow data when available, and highest levels of the five pharmaceuticals that could be measured by the analytical methods available for water and fish (carbamazepine, metoprolol, hydrochlorothiazide, sertraline and atenolol). Results indicate that several pharmaceuticals are found in fish fillets in Urban systems and where pharmaceuticals are detected in water. The views expressed in this presentation are those of the authors and do not represent those of U.S.EPA.

299

Bensodiazepines; bioconcentration and bioaccumulation in various biota and presence i European surface waters.

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In a recent study we showed that an anxiolytic drug, the benzodiazepine Oxazepam, alters behavior and feeding rate of wild European perch *(Perca fluviatilis)* [1]. In this study only a single species was investigated but inter-species internal levels, following exposure of a pollutant, may vary due to diverse bioaccumulation rates. This will of course have implications in effluent dominated aquatic environments since not all exposed biota present will be affected at the same exposure levels. In addition, not all species have the mode-of-action targets for pollutants, which are relevant in the case of pharmaceuticals, since the environmental effects would have to be correlated with conserved targets in exposed biota and not with a general toxic effect [2]. Benzodiazepines, for examples, act upon the γ-aminobutyric acid (GABA) receptor and several species have orthologs of this receptor [2]. This study is focused on bioconcentrations factors (BCFs) and bioaccumulation (BAFs) factors of one of the besodiazepines, oxazepam, in Perch, Crucian carp, Sticklebacks, Damselfly larvae. Dragonfly larvae, Caddisfly larvae and Mayfly larvae. By investigating BCF and BAF in several species we can describe the flow of benzodizepines in the food web. What makes this especially interesting is the fact that several of the studied species do not have the GABA receptor and therefore will most likely not be affected at environmental relevant levels and that different species have larges differences in bioconcentrations factors. Damselsfly larvae are, for example, not affected by oxazepam at water concentrations (1.8 microg/L) that affects Perch, even though our studies show that they bioconcentrate 120 times more (compared to Perch). That some invertebrates have elevated bioconcentration factors makes bioaccumulation (BAFs) factors highly relevant to study and we will present results from several predator-prey studies using Perch and Dragonfly larvae as predators and Sticklebacks, Damselfly larvae, Caddisfly larvae and Mayfly larvae as prey. We will also present the results from an EU-wide study of the presence of 13 bensodiazepines in surface water. Four European River catchment systems, River Aire-Calder, Blanice River, River Tiber and the Danube were included (17 rivers and tributaries in 9 countries). 1. Brodin, T, et al. *Science* **339,** 814–815 (2013). 2. Gunnarsson, et al. **42,** 5807–5813 (2008).

300

From theory to reality – Evaluation of suitable organisms and test systems for the biomonitoring of pharmaceuticals - Literature review

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A vast diversity of human and veterinary pharmaceuticals is frequently detected in aquatic ecosystems. However, the actual effect of these substances on biota is hardly assessable. Since the principle of pharmacovigilance also demands the examination of environmental effects, it is crucial to develop new, effect-based strategies for the monitoring of pharmaceuticals. As a first step, we conducted a survey of recently published literature (2011 to 2013) to gain an overview, which pharmaceutical groups appear to be of highest relevance for biota, and which organism groups and effect endpoints react most sensitively to pharmaceuticals. A database was created from the given information and the studies were evaluated with respect to the reported lowest observed effect concentrations (LOECs), which were subsequently compared to measured environmental concentrations (MECs) in surface waters. Additionally, we investigated the reliability of studies reporting LOECs in close range to the MECs. Overall, 452 studies were examined, of which 232 directly investigated pharmaceutical effects. The most frequently studied organism group were fish, with *Danio rerio* being the most commonly used species, followed by molluscs. It was also shown that a huge variety of different species is applied in the evaluation of pharmaceutical effects, but only few of them are investigated in more than one study. Considering relevant endpoints, behaviour, vitellogenin synthesis, growth, reproduction and histopathological changes were among the most sensitive responses. Based on the effect information extracted from the studies, the analgesic diclofenac, the β-blocker propranolol, the lipid regulator gemfibrozil and the antibiotics erythromycin and sulfamethoxazole were identified as environmentally most relevant pharmaceuticals. Unfortunately, the evaluation also showed that only a minor part of the investigated publications can be considered as fully reliable; most studies included at least some minor flaws. Furthermore, there still appears to be a huge knowledge gap considering the effects of mixtures, and the population relevance of many observed effects remains unclear. For further monitoring strategies, we propose a system based on effect classes rather than leading substances, and want to stress out the crucial need for mode-of-action based testing.

Macro, micro and nanoplastic pollution in the aquatic and terrestrial environments: Sources, fate, exposure

and ecological and toxicological impacts

301

Releasing of hexabromocyclododecane (HBCD) from expanded polystyrene (EPS) buoy in marine water: a field study

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Polystyrene is widely used as building insulation material, floating device and in packaging. It is also a major component of debris found to in-land and marine system. To prevent it's burning, HBCD is frequently added as flame retardant. HBCD is an additive, not covalently bonded to the material, leading to the risk of migration out of the product during production, use or disposal. Due to its persistence and adverse biochemical effects, it has been added to the list of global elimination compounds under the Stockholm Convention in May 2013. However, the interesting point is the presence of HBCD in water, oyster and sediment collected from and nearby aquafarms i.e. non-industrial area. EPS buoy, a rich source of HBCD (~70-75 μg/g of EPS), is highly used in Korea for the culture of longline oyster (2 million buoys/year). It may be considered as a cause of HBCD contamination through leaching by sea water. In view of this, it is imperative to carry out a systematic field study on the leaching of HBCD from EPS buoy in sea water. Studies in the field was designed in enclosed-rectangular-glass chambers (50x100x50cm) and mesocosm. A monolayer of detached EPS spherules was dispersed on the surface of water. The leaching of HBCD was investigated by determining the residual HBCD in known amount of EPS collected at different interval of time.Results show that there is an exponential decrease in the concentration of HBCD. Leaching characteristics that fast-leaching initially followed by slow desorption over time were generally observed. Nevertheless, γHBCD was the most abundant isomer followed by αHBCD and βHBCD, of the total HBCD concentration. The lower α/γ ratio (0.22, Fresh EPS: 0.35) of HBCD isomers observed in collected EPS favoured the solubility of αHBCD. Among three conditions investigated in the field for two months (August and September), fastest kinetic was observed in mesocosm followed by sun- and dark-exposed condition highlighting the phenomenon of weathering and degradation. Within 3 days, the percentage of HBCD lost in laboratory and field is 20 and 77%, respectively. In field test, the maximum leaching rates were estimated to reach in range of 53 to 86% (from dark exposure to mesocosm) which were significantly affected by high volume of water, UV light, microorganisms, physical impact and temperature. Results obtained during the study indicate that EPS buoy is a significant source of HBCD in Korean marine environment.

302

Enrichment of additive HBCDs in mussel attached to expanded polystyrene buoy

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Expanded polystyrene (EPS) is widely used polymer for varying purposes including insulation materials, packing materials, and aquaculture buoy. Large amount of EPS buoys has been used for aquaculture of oyster and mussels in South Korea leading to a large number being lost or disposed as waste. Floating EPS buoy as marine debri have become a habitat for marine organisms. In our previous study, we detected considerable amounts of hexabromocyclododecanes (HBCDs) in EPS buoy with a median concentration of 40 μg/g. To find evidence on marine debris as a source for toxic chemicals to marine organisms, floating EPS buoy and attached mussels were collected from Jinhae Bay, Korea were chemically analysed. Samples were categorized as follows; EPS buoy, mussel attached to its EPS buoy (Group 1), mussels attached to high density polyethylene (HDPE) buoy (Group 2), mussels attached to non-polymeric substrate (Group 3). The concentration of HBCDs in EPS buoy (μg/g dw)and Group 1 mussel (μg/g lipid weight) were in the range of 0.15-1,580 (mean; 246±571) and 0.074-5.16 (1.3±2.19), respectively. The HBCD concentration among EPS buoys and mussels showed large variations. However, HBCDs in mussel were positively related to HBCDs in EPS they were attached, indicating HBCD level in individual buoy might directly affect the mussels on it. Mussels collected from HDPE buoy (Group 2) and non-polymer substrate (Group 3) accumulated much smaller amounts of HBCDs than that in Group 1 with the concentration range of 0.062-0.097 μg/g lipid weight (mean; 0.07±0.015) and 0.035-0.062 μg/g lipid weight (0.056±0.015), respectively. Although the distance between EPS and HDPE buoys was less than 1m, approximately 5 times higher concentration of HBCDs was detected in mussels from EPS buoy than in HDPE buoy. There were clear changes in α/γ ratio in mussels according to the type of their substrates. In this study, we tried to find evidence on raising questions of contributions of macro- and micro-sized plastic marine debris bioaccumulation to marine life through analysis of mussels attached to EPS substrate collected. The body residues and isomeric profiles of HBCDs in mussel reflect those in their plastic substrates. This result strongly implies that plastic marine debris can be a moving source of toxic chemicals to marine life and can be biomagnified in marine food chain.

303

Microplastic particles in aquatic sediments: New methods for sampling, extraction and quantification

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Although plastic-debris is constantly accumulating in aquatic-environments, the impact on aquatic-ecosystems is not yet fully understood. A first important step in order to assess the consequences of plastic-debris in aquatic-ecosystems is the establishment of a reliable, verified and standardized method to quantify the amount of plastic-particles of all ecologically relevant size classes (down to 1μm).\n In this study we developed a novel density separation setup (Munich Plastic Sediment Separator -MPSS) which allows for a successful separation of mesoplastic particles (20–5 mm), L-MPP (5–1 mm) as well as for S-MPP (Validation of the MPSS was done using the most commonly used plastic types, including two with relatively high specific densities. Identification of the found particles was performed by Raman microspectroscopy (RM). The new method was compared with the "classical density separation setup" comparable to other studies. Moreover we improved the sampling of aquatic beach sediments by combining the advantages of transect sampling and sediment core sampling. This results in a representative sample of one beach segment. With a volume which perfectly fits into the newly developed MPSS. Additionally we suggest certain criteria for transect placement to enable realistic and representative sampling and subsequent quantification of microplastic particles This is to our knowledge, the first study which examined systematically the recovery rates of different separation methods and provides a standardized apparatus for the process of density separation complying all requirements for the extraction of even very small particles (down to several microns) from sediment samples. For the identification and quantification of the plastic particles in sediments, RM can be successfully applied. In combination with other separation liquids the MPSS and RM could also be applied for the separation and identification for any particles down to the micrometer range from a sample with high volume (up to 6 l). It perfectly suits the requirements in handling microparticles and could also be constructed on a smaller scale.\nThe evaluation of the suggested sampling method revealed a representative location for beach transect. The use of sediment cores proved to be useful in combination with the use of the MPSS.

304

Microplastics in field collected and cultured bivalves

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Microplastics are present throughout the marine environment, and ingestion of these small plastic particles has been demonstrated repeatedly in a laboratory setting for a wide array of marine organisms. However, as the exposure concentrations of microplastics in these types of experiments is much higher than any reported field concentration, it is difficult to assess the relevance of these results for natural environments. Therefore, we assessed the presence of microplastics in two bivalve species living in natural conditions: both field collected and cultured individuals of *Mytilus edulis*, and farmed *Crassostrea gigas* were investigated. Part of the organisms (all field collected *M. edulis* (N=30), and half of the cultured *M. edulis* (N=36) and *C. gigas* (N=10)) were subjected to a gut depuration to allow them to clear their gut. To examine microplastic presence, the soft tissues were acid digested. Small numbers of microplastics were recovered from the tissue of the species under investigation. In field collected *M. edulis* the average microplastic load (post-depuration) was 0.20 ± 0.30 particles per gram of soft tissue. In cultured *M. edulis* microplastic load (pre-depuration) was 0.36 ± 0.07 particles.g^{-1} of soft tissue. After the depuration period, only 0.24 ± 0.07 particles.g^{-1} were encountered. The same trend was observed in *C. gigas*: without depuration on average 0.47 ± 0.16 particles.g^{-1} were present in the animals, while microplastic concentrations decreased to an average of 0.35 ± 0.05 particles g^{-1} after depuration. It is not surprising that the microplastic load of cultured animals resembles that of field collected animals since they are cultured in natural conditions. As a result these filter feeding organisms are exposed to any pollutant present in the seawater, including microplastics, in the same way as their wild counterparts. Although it is now established that mussels and oysters contain microplastics, this is the fist report so far on microplastics in foodstuffs. Currently, only a preliminary dietary exposure could be estimated. The hazard posed by microplastics needs to be established through in-depth toxicological studies. Due to a lack of dedicated studies, the complexity of estimating particle toxicity hinders a comprehensive assessment of the risks associated with microplastics. Estimations of the potential risks for human health posed by microplastics in food stuffs is not possible.

305

Nanoplastic affects growth of S. obliquus and reproduction of D. magna

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The amount of nano- and microparticles in the aquatic environment rises due to the industrial production of nanoplastic and the degradation of macroplastic into small particles. Little is known about the fate and effects of nanoplastic, while there are lots of speculations about possible effects. In this study, the effects of nano polystyrene on performance of green algae *Scenedesmus obliquus* and zooplankton *Daphnia magna* were assessed. At high doses inhibiting effects on the growth of *S. obliquus* were shown. During chronic tests the suspensions of nano polystyrene were not lethal to *D. magna* but reproduction effects were observed. Interestingly, aqueous vs. dietary exposure to nano polystyrene played an important role in the occurrence of effects on *D. magna*. Thereby this study provides a novel indication about the importance of uptake routes in nano plastic exposure.

306

Tracing POP-BDE routes through plastic waste streams in the Netherlands

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There is currently only limited understanding of the dynamics of the distribution and levels of tetra-, penta-, hexa- and heptabrominated BDE congeners (POP-BDE) in waste streams in Europe and worldwide. This study investigated how plastic waste materials that may contain POP-BDE are sorted, separated, disposed of, recycled, landfilled, incinerated and/or exported in the Netherlands. This was coupled to measured concentrations of POP-BDEs in samples taken from four points in the waste streams to study the mass flow of these regulated POPs through the Dutch plastic cycle. A cost-effective, fast 'direct probe' screening method developed at IVM was applied to quickly determine the presence or absence of POP-BDEs. The method makes use of a direct probe coupled to atmospheric pressure chemical ionization-high resolution time-of-flight-mass spectrometry (APCI-HRTOF-MS). The method can be used to screen samples for POP-BDEs in plastic, so that the more laborious solvent extraction procedures are only done when quantifiable amounts are present. In total 90 samples were selected for determination of the POP-BDE concentrations, using gas chromatography with the electron capture negative ionization technique and mass spectrometry detection (GC/ENCI-MS), using a highly sensitive method. This study provides a unique POP-BDE dataset for the relevant plastic waste streams in the Netherlands. POP-BDEs were rarely found in single automotive parts (when detected, it was only in car parts from the USA) or WEEE items. Shredder material (consisting of a large number of shredded items), frequently did contain POP-BDE. The samples in which POP-BDEs were detected confirmed that c-PentaBDE can be found in automotive, whereas the c-OctaBDE pattern is found in WEEE. DecaBDE (not a Stockholm POP) is frequently found in plastic fractions from shredded automotive and WEEE material and recycled plastic pellets. Based on the mass flow analysis, 22% of the POP-BDE in WEEE is expected to end up in recycled plastics. In the automotive sector, 14% of the POP-BDE is expected to end up in plastics recycling, while an additional 19% is expected to end up in second-hand parts (reuse). POP-BDEs were detected at low concentrations in some new products made from recycled plastic (imported products). This also indicates that the legacy of POP-BDEs will be with us for quite some years to come, until the plastics are downcycled to the point that they are sent for incineration.

Recent advances and critical future research directions for poly- and perfuorinated alkyl substances (PFASs) (I)

307

Global emissions, fate and transport of C6-C10 perfluoroalkane sulfonic acids (PFSAs): what we know and what we do not know

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Long-chain perfluoroalkane sulfonic acids (PFSAs, $C_nF_{2n+1}SO_3H$, $n \geq 6$), especially perfluorooctane sulfonic acid (PFOS), are a group of global contaminants of high concern. These substances are highly persistent, bioaccumulative, toxic, and distributed ubiquitously in the environment, biota, and humans. A recent study estimated a global emission inventory of PFOS and PFOS precursors (PreFOS) and coupled the emission inventory to a global-scale multimedia mass-balance model to assess the global fate and transport of PFOS and PreFOS. That work highlighted the significance of direct industrial sources to the overall distribution of PFOS in the global environment. However, that work also demonstrated that the estimated global emissions of PreFOS are too low by one to two orders of magnitude. Moreover, that study did not realistically represent the recent production of PFOS and PreFOS in China. Furthermore, previous studies focused solely on PFOS and PreFOS, whereas their short- and long-chain homologues have also been produced since the 1960s. To tackle these knowledge gaps, we use new information on PFSA uses and disposal and develop a new global emission inventory of C_6–C_{10} PFSA homologues from 1958 to 2030. Then we use a global-scale multimedia mass-balance model to assess the global fate and transport of these PFSAs in the environment. We estimate that 2700–7000 tonnes of PreFOS and 3600–6000 tonnes of PFOS are emitted between 1958 and 2015. Our new estimates are higher than the previous estimates. In particular, we estimate one to two orders of magnitude higher emissions of PreFOS, which is enough to close the mass balance between emissions of PreFOS and their levels measured in the global atmosphere. Overall, our estimates of the direct emissions of PFOS from production sites and during the product use and disposal phases are much higher than the indirect emissions of PFOS from degradation of PreFOS. Furthermore, we highlight several critical knowledge and data gaps. For example, we estimate that about 800 tonnes of perfluorooctane sulfonyl fluoride (POSF) have been emitted since the 1950s. However, due to a lack of reliable degradation half-life data for POSF in air, it is not possible to estimate the time-resolved historical contribution of this additional source to the environmental levels of PFOS.

308

Occurrence and removal fluxes of Perfluorinated alkyl substances in the tropical and subtropical Global Oceans.

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Perfluorinated alkyl substances (PFASs) are receiving an increasing worldwide attention due to their persistence, toxicity and widespread occurrence. These emerging contaminants have high water solubility and lower lipophilicity compared to legacy persistent organic pollutants, being their global fate and removal processes still uncertain due to their particular chemical characteristics. This study provides the global concentrations of three PFASs families, perfluorinated carboxylic acids, perfluorosulfonates and perfluorosulfonamides, in surface seawater and at deep chlorophyll maximum depth in the global tropical and subtropical oceans. Horizontal and vertical occurrence was evaluated in north and south basins of Atlantic and Pacific Oceans and in the Indian Ocean during the Malaspina 2010 circumnavigation cruise. The Atlantic appeared as the most polluted ocean in our global study; followed by the Pacific and then the Indian Ocean. Mean ΣPFASs concentrations found in the Northern hemisphere were lower than values found in the Southern hemisphere, suggesting differences in emission patterns in both Hemispheres. It is remarkable the high relative abundance of perfluorooctanoic sulphonate (PFOS) and perfluorooctanoic acid (PFOA). Removal fluxes due to vertical turbulent diffusion in the water column and to sorption to biomass and sinking are considered the two main removal processes affecting the superficial concentrations of PFASs as biodegradation has not yet been reported for these compounds. The oceanic sink due to vertical eddy diffusion is given for individual PFASs. Turbulent fluxes were estimated with a 1D diffusion model and using KT (eddy diffusion coefficient) measured simultaneously to the PFASs concentrations during the Malaspina cruise. In addition, the sinking flux (biological pump) can be predicted from the dissolved phase concentrations, the fluxes of organic carbon estimated from measured chlorophyll a concentrations, and reported bioconcentration factors (BCF) for marine phytoplankton. Modeled fluxes suggest that the biological pump is far more efficient in removing PFASs from the surface ocean than the turbulent flux; being the latter only relevant during extreme mixing events. However, both removal fluxes are low (less than 0.01 ng m^{-2} d^{-1} for PFOA and PFOS) suggesting long residence times of PFASs in the surface ocean. Other parameters as sources, vicinity to coast or ocean currents also affect the global distribution of PFASs.

309

Ecodynamics of selected PFASs in the Gironde estuary

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Over the past 15 years, there has been a flurry of research activities in the analysis of poly- and perfluoroalkyl substances (PFASs) in water, sediments and wildlife, due to their persistent, bioaccumulative, and toxic properties. However, little is known about their ecodynamics, especially in estuarine ecosystems. In this work, the Gironde estuary was selected as the study site because of its ecological value: it

is home to the largest assemblage of migratory amphihaline fish in Western Europe. Water, sediment and a wide range of biota samples were collected at selected locations in this estuary. Biota samples included benthic invertebrates, zooplankton (copepods, mysids), shrimps, and several fish species – grey mullet, meagre, seabass and sole. The contamination of biota was therefore assessed for top predators but also for several taxa at the bottom of the food web, whose PFAS contamination remains poorly documented. In view of the high number of values that fell below detection limits, statistical treatments taking into account non-detects were implemented, leading to more accurate calculation of descriptive statistics, correlations, and differences among groups. The relationship between the contamination of biota and that of abiotic compartments was quantitatively assessed: bioaccumulation factors and biota to sediment accumulation factors were calculated. Biomagnification factors (BMF) and trophic magnification factors (TMF) were also determined to quantify PFAS transfer within the food web. In addition, a specific statistical modelling approach is currently implemented to evaluate the relative influence of trophic position and biometric characteristic on PFAS bioaccumulation in key fish species (common sole and spotted seabass).

310

Temporal Trend of Perfluoroalkyl Compounds in the Atmosphere at Dalian, China: Evidence of Unknown PFC?

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The level and profile of perfluoroalkyl compounds (PFAAs) in the atmosphere indicate their local distribution and the global transportation. However, there are still only a limited number of studies to determine atmospheric concentrations of PFAAs.Dalian is a coastal city located in Liaoning Province, northeast of China. The city, as well as other regions in Liaoning, has been reported of higher PFAAs concentrations, in water, sediments, aquatic organisms, and even human serum, than most of other regions in China, by both our previous studies and other researches. Therefore, the environmental risk of PFAAs in this area has attracted extensive concern of both the scientists and the public. The purpose of the present study is to trace the trend of PFAAs levels and profiles in the atmosphere in this region. Particulate matters were collected at Dalian, in 2006-2013, and the PFAAs concentrations were reported. Three perfluorosulphonates (C4, 6, 8) and eight perfluorocarboxylates (C5-12) were dected by HPLC-MS/MS, where internal standard calibration was employed for quantification. Furthermore, the unknown PFC was analyzed, to evaluate the impacts of the replacement in fluorine chemistry on the environmental pollution.

311

Factors Impacting Groundwater Fate and Transport of Perfluoroalkyl Acids at AFFF-impacted Sites

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The recent implementation of soil and drinking water regulatory guidance values for some perfluoroalkyl acids (PFAAs) reflects growing concerns regarding the presence of these persistent and bioaccumulative chemicals in the environment. Because of their unique properties, PFAAs have a wide variety of uses including aqueous film-forming foams (AFFF). AFFF is used to extinguish hydrocarbon fuel fires by fire departments, the hydrocarbon industry, and the military. Concentrations of PFAAs in groundwater have been measured in the ng/L - µg/L range at fire training facilities where AFFF was repeatedly used in training exercises. These sites may also be impacted by co-contaminants including non-aqueous phase liquids (NAPL). Where co-contaminants are present, site conditions may also be impacted by previous remedial efforts such as in situ chemical oxidation. Thus, the present work invesitages the transport and reaction processes for 10 PFAAs in the subsurface by evaluating batch sorption and 1-dimensional column transport in the presence and absence of co-contaminants and chemical oxidants. Batch sorption experiments demonstrate that sorption of smaller PFAAs does not follow a chain-length dependent trend with sorption of perfluorobutanoate(PFBA) similar to that of perfluorooctanoate (PFOA). The primary impact of NAPL was an increase in isotherm linearity leading to a concentration-dependent impact to sorption Preliminary results suggest that sodium persulfate may increase PFAA sorption due to decreases in system pH in the presence of this oxidant. Column breakthrough of PFAAs yielded column sorption partitioning coefficients (K_d) values smaller than those measured in equilibrium systems, indicating a potential for nonequilibrium transport. Lastly, Flow interruption experiments verified that nonequilibrium tranport was caused by rate-limited sorption of some PFAAs. PFAA breakthrough curves were modeled to obtain rate constants on the order of 10^{-7} to 10^{-5} min^{-1} that can be incorporated into a 2-site sorption model to describe nonequlibrium, advective PFAA transport. These results demonstrate a need for additional studies of the fate and transport of PFAAs, in particular research that further explores the impact of co-contaminants and oxidants in advective scenario.

312

Prediction of Partition Coefficients of Per- and Polyfluoroalkyl Substances and Organosilicons using Polyparameter Linear Free Energy Relationships

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Per- and polyfluoroalkyl substances (PFAS) have received an increasing attention over the past decade. PFAS have characteristic partitioning properties such as relatively high volatility and hydrophobicity compared to the hydrocarbon-based analogues. These properties occur due to the very weak van der Waals (vdW) interaction properties of PFAS. Such characteristic vdW interaction properties are partially shared by organosilicon compounds, another compound class of recent environmental interest. Property estimation models that are calibrated only with hydrocarbon-based compounds can give rise to large errors in estimated values of PFAS and organosilicons, because the latter compounds are out of the model application domains. In this study, polyparameter linear free energy relationships (PP-LFERs) were evaluated for their accuracy to predict environmentally relevant partition coefficients of neutral PFAS and organosilicon compounds, using fluorotelomer alcohols (FTOHs) and cyclic volatile methyl siloxanes (cVMS) as test chemicals. Literature data for octanol–water, air–water, octanol–air, and organic carbon–water partition coefficients of FTOHs and cVMS were used for model validation. To amend the data set, olive oil–water and liposome membrane–water partition coefficients of FTOHs were determined. The results show that, in most cases, the PP-LFER predictions for FTOHs and cVMS are within 1 log unit of the experimental data. In general, a recently proposed form of PP-LFER that uses molar volume (V) and the log hexadecane-air partition coefficient (L) as descriptors for non-specific interactions provided more accurate predictions than the traditional forms of PP-LFERs. The traditional PP-LFERs even caused > 2 log-unit errors for predictions of air-water partition coefficients. A further optimization of descriptors and parameters resulted in high agreement between experimental and PP-LFER-calculated partition coefficients (RMSE < 0.2 log units), if the newer form of PP-LFER was used. The results of this study indicate that the newer form of PP-LFER that uses V and L has significant advantages over the former PP-LFERs to describe and predict the partitioning behavior of PFAS and organosilicons.

Usage, fate and risk of carbon based nanomaterials

313

Strong sorption of PCBs to carbon nanotubes, fullerenes, nanoplastics and micorplastics. Effects of salinity and sediment organic matter.

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With the presence of carbon-based nanoparticles and microplastics in the environment, implications for the fate and effects of traditional hydrophobic chemicals are considered. Therefore, a comparative study was done on the sorption of 17 PCBs to 2 carbon nanoparticle types: multiwalled carbon nanotubes (MWCNTs) and fullerene (C_{60}), 2 types of plastics: micrometer sized polyethylene (micro-PE), nano-polystyrene (nano-PS) and a natural sediment. To increase environmental realism and assess the effects of salinity and sediment organic matter (OM) fouling, multi-solute sorption isotherm experiments at 10^{-5} to 10^{-1} µg L^{-1} were performed in fresh- and seawater, with and without the presence of sediment. Sorption to the 'bulk' sorbents OM and PE dominated by linear hydrophobic partitioning with OM and PE having similar sorption affinity where sorption to MWCNTs, C_{60} and PS was non-linear. PCB sorption to MWCNTs and C_{60} was 3-4 orders of magnitude higher than to OM and PE. Sorption to 70 nm PS was 1-2 orders of magnitude higher than to 10-180 μm PE, which was related to the higher surface-volume ratio of nano-PS. OM and salinity effects varied among sorbents with the largest OM fouling effect observed for the high surface sorbents (MWCNTS, nano-PS), and salinity decreasing sorption for sediment and MWCNTs, but an increasing sorption for the polymers nano-PS and PE. The exceptionally strong sorption of (planar) PCBs to C_{60}, MWCNT and nano-PS in combination with their capability to penetrate membranes may constitute an unforeseen risk.

314

Biological Uptake and Depuration of Radio-labeled Graphene by Daphnia magna

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Graphene layers are potential candidates in a large number of applications. However, little is known about their ecotoxicological risks. One critical factor is the extent to which graphene bioaccumulates in organisms and spreads through food chains. However, this topic has not been investigated yet, largely because methods have not been available to readily quantify it in complex environmental or biological systems. In this study, graphene was synthesized by means of graphitization and exfoliation of sandwich-like $FePO_4$/dodecylamine hybrid nanosheets, and ^{14}C was incorporated in the synthesis (see Figure 1). Carbon-14-labeled graphene was spiked to artificial freshwater and the uptake and depuration of graphene by *Daphnia magna* were assessed. After exposure for 24 h to a 250 μg/L solution of graphene, the graphene concentration in the organism was nearly 1% of the organism dry mass. These organisms excreted the graphene to clean artificial freshwater and achieved roughly constant body burdens after 24 h depuration periods regardless of the initial graphene exposure concentration. Addition of algae and humic acid to water during the depuration period resulted in release of a significant fraction (>90%) of the accumulated graphene, but some still remained in the organism. Accumulated graphene in adult *Daphnia* was likely transferred to the neonates. The uptake and elimination results provided here support the environmental risk assessment of graphene and the graphene quantification method is a powerful tool for additional studies.

315

Prediction of sorption of organic compounds by multi-walled carbon nanotubes using poly-parameter linear free-energy relationships

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The interpretation and prediction of molecular interactions between organic compounds (OC) and carbon-based nanomaterials (CNM) is of major importance to understand the phase transfer processes in environmental matrices. Sorption of OC by CNM has already been studied to some extent; however, time consuming batch experiments in combination with the variability of OC make it impossible to determine all distribution coefficients (K_d) in laboratory experiments. The correlation between K_d values of OC and their physicochemical properties (e.g., K_{ow}) is common practice in environmental chemistry; however, for the prediction of sorption these correlarions are only valid for the compound class and solvent/sorbent phase, which were used for model development. These disadvantages may be overcome by the use of poly-parameter linear free-energy relationships (ppLFER), which incorporate the contribution of individual molecular interactions by properties of the sorbate and the sorbent in a single equation [1]. Recently, a ppLFER model was reported for sorption of aromatic compounds by MWCNTs [2]. However, not all terms of the ppLFER were considered in the development process, which may be necessary for a comprehensive discussion of the relevant interactions. In addition, the derived phase decriptors depend the calibration range of the compound set used for ppLFER development. Thus, the aim of this work was to derive a ppLFER for sorption of multi-walled carbon nanotubes (MWCNTs) in order to investigate the contribution of individual molecular interactions to overall sorption. To this end, individual sorption isotherms of 37 compounds were experimentally determined in batch experiments. The sorbates covered a broad range of substance classes, e.g., non-, mono, and bipolar aliphatic and aromatic compounds. The here presented data set extends literature data by the incorporation of aliphatic compounds, which leads to an overall better prediction of sorption, especially for aliphatic compounds. The results showed that the development of a ppLFER equation allowed a precise prediction of sorption of OC by MWCNTs. The contribution of individual molecular interactions to overall sorption was evaluated, by which the sorption properties of MWCNTs could be characterization using the ppLFER approach. [1] Abraham MH. 1993. Chem Soc Rev 22:73-83. [2] Apul OG, Wang Q, Shao, T., Rieck, JJ, Karanfil T. 2013. Environ Sci Technol 47:2295-2303.

316

Nanotraces in the nanoworld – Occurrence of fullerenes in environmental samples

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Fullerenes are carbon based nanomaterials in a hollow-spherical shape which, during the recent years, have been extensively studied. While some relevant aspects of their behaviour, fate and their ecotoxicity effects start to be well understood, their quantification in real environmental samples is still an analytical challenge, mostly because of their extremely low concentration levels. In comparison with other emerging contaminants, very few works have reported the occurrence of fullerenes in the environment although characterizing their concentrations in several environmental compartments is an important task for properly assessing the real environmental risks of fullerenes. In this work, several types of environmental matrices (river water, wastewater, soils and sediments) have been analyzed by HPLC, with buckyprep columns, coupled with an atmospheric photoionization source to a high resolution mass spectrometer with a hybrid quadrupole-orbitrap analyzer. This instrumentation offers unique sensitivity and selectivity and the method performance allows the detection of fullerenes in the low pg/L order in water samples. Surface water and sediments samples from two river basins from Barcelona under high anthropogenic pressure have been analyzed. As expected, the release of wastewater effluents is observed to increase the concentrations of unfunctionalized fullerenes in some hot spots. These results and the possible origins of fullerenes will be further discussed. Finally, the occurrence of fullerenes in soils has been studied. Fullerenes have been reported to be emitted from combustion and industrial sources and, in previous works, they were detected in ppq-ppqt concentrations in atmospheric particulate and soils from Saudi Arabia, near oil refinery plants. In the present work, we report the results of the analysis of soils located near fossil combustion power station in Brazil.

317

Effects of co-exposure of single walled carbon nanotubes and ethinyl estradiol on estrogen receptor binding, activation, and downstream responses in fish

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Single walled carbon nanotube (SWCNT) use in manufacturering has led to concerns that these materials may become contaminants of environmental concern. Previous research in our laboratory has shown that SWCNTs are not acutely toxic and are not readily taken up through intestinal exposure routes. But the potential for interaction with other environmental contaminants has lead our laboratory to consider other mechanisms of toxicity for these materials. For example, researchers have found that SWCNTs will readily bind ethinyl estradiol (EE2), a potent synthetic estrogen, potentially decreasing its bioavailability. Inadquate removal during wastewater treatment has led to measureable concetrations of EE2 in final treated wastewater effluent and receiving waters. Fish exposed to EE2 at environmentally relevant concentrations have been shown to exhibit reproductive disruption and evidence of similar effects in the wild suggest that EE2 may be causing reproductive toxicity in the environment. The objective of the current study was to examine the interaction and effects of SWCNTs and EE2 mixtures using a number of *in vitro* and *in vivo* molecular techniques. Fluorescence polarization binding assays and luciferase reporter assays were used to characterize the effect of SWCNTs on the binding and activation of the estrogen receptor (ER) by EE2, respectively. To compare *in vitro* and *in vivo* responses, largemouth bass were gavaged with EE2 and EE2/SWCNTs mixtures. Livers were analyzed for nuclear ER and VTG gene expression levels using qPCR as well as differential protein profiles using iTRAQ LC-MS/MS based proteomics techniques. SWCNTs decreased in vitro binding of EE2 to the estrogen receptor. Luciferase reporter assays showed that in addtion to binding, activation of the estrogen receptor by EE2 is also decreased in the prescence of SWCNTs. The *in vivo* gavage study is currently underway to determine if our *in vitro* responses translate to transcript and protein effects in whole organisms. Results from this study will help increase our understanding of how SWCNTs will interact with the complex mixture of contaminants in our environment as well as natural hormones in organisms.

318

Sorption behaviour of carbon-based nanomaterials: effects of dispersion and aging

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Understanding the interactions between organic contaminants and carbon based nanomaterials (CNMs) is essential for evaluating the materials' potential environmental impact as well as the potential efficiency as superior sorbents. Although a great deal of work has been published on the subject, sorption data remain limited in terms of compounds, concentrations, and conditions investigated. A passive sampling method was developed and validated [1] to address a number of important knowledge gaps on a series of carbon nanotubes [1,2] and fullerenes (C60) [3]. The presentation will combine the results obtained for a series of PAHs and over a range of dispersion scenarios that CNMs may undergo during their life cycle. We considered both intentional dispersion events (e.g., sonication, addition of surfactants) and environmental aging events (e.g., presence of oxygen, natural organic matter, irradiation). Sorption isotherms across a wide range of concentration and extensive characterization of the CNMs systems were combined in order to support mechanistic interpretations of the results. Overall, the series of studies shows that the effects of dispersion and aging cannot be neglected when studying interactions of organic contaminants and CNMs (i.e. discrepancies of orders of magnitude were observed in some cases). We also demonstrate that adaptation of classical experimental set up is necessary to generate reliable data, and evaluate the potential applications and environmental impact of CNMs. [1] Kah M, Zhang XR, Jonker MTO, Hofmann T. 2011. Environ Sci Technol 45: 6011-6017. [2] Zhang XR, Kah M, Jonker MTO, Hofmann T. 2012. Environ Sci Technol 46: 7166–7173. [3] Hüffer T, Kah M, Hofmann T, Schmidt, TC. 2013. Environ Sci Technol 47 : 6935-6942.

Modelling of chemical fate and exposure in a regulatory context (III)

319

GERDA: A new exposure approach for pesticide inputs into surface waters via surface runoff, erosion and drainage in Germany

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The German national registration procedure currently uses the model EXPOSIT 3.01 to evaluate the surface water exposure from pesticides input with runoff, erosion, and drainage. To adjust the German national exposure and risk assessment procedure to the FOCUS surface water approach and furthermore to enhance some of the its limitations and shortcomings the German Federal Environment Agency (UBA) has launched in 2011 the development of GERDA (GErman Runoff, Erosion, and Drainage risk Assessment) which probably will replace EXPOSIT in future. The concept and the main components of the GERDA project are presented. The models PRZM and MACRO are still used to assess the edge-of-field pesticide losses. Instead of TOXSWA, GERDA uses the model STEPS-1-2-3-4 to calculate PEC hourly time series in the receiving water body. A 30-year time series is applied to assess PECsw/PECsed, in contrast to only 12 or 16 months in FOCUSsw. The ecotox effects of different concentration profiles has been captured and ranked by TK/TD modeling. Results show that both maximum PEC and the AUC (area under curve), are highly rank-correlated to the modeled ecotox effects. Thus both descriptors are used to create cumulative distribution functions (CDFs) which allow identifying scenarios protective for a specific proportion of the landscape. A reliable *a priori* assessment of the "worst-case-ness" of soil-climate combinations is not possible. For a specific soil-climate scenario, defined by a weather time series and a bundle of soil properties, the percentile of the edge-of-field loss calculated by PRZM and MACRO, respectively, can not be derived from the cumulative distribution functions (CDFs) for a soil properties and/or climate variables. To select soil-climate scenarios in a rigorous, statistically based manner, 350000 PRZM and 90000 MACRO simulation runs for soil/climate/crop/substance-combinations, covering the entire arable land area in Germany, were used to calculate long-term PECsw time series. The time series were ranked according to the calculated PECsw to create area-weighted CDFs for Germany. Details on the derivation of the specific national soil-climate scenarios (soil classification and climate zone clustering) have already been presented. This approach facilitates the identification of soil-climate scenarios with a given cumulative probability with respect to ecotoxicological effects and the spatial representativity of the selected soil-climate scenarios.

320

GERDA: A new software tool for pesticide exposure assessment for surface waters in Germany

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The EU regulation (EC) No1107/2009 calls for a harmonization of the various national pesticide exposure and risk assessment approaches between EU member states. The German Federal Environment Agency (UBA) therefore launched a project (FKZ 3711 63 427) to harmonize the German national exposure assessment procedure for surface waters with the FOCUSsw scenario approach used at the EU level for approval of active substances. Within the project the exposure assessment tool GERDA (German Erosion, Runoff and Drainage Assessment) has been developed which takes into account the full range of agro-pedo-climatic conditions in Germany. GERDA produces 30-year PECsw/sed time series for i) drift and drainage inputs and ii) drift, surface runoff and erosion (and potentially lateral subsurface flow) inputs into surface water. Pesticide losses from the field via tile drains (and lateral subsurface flow) are simulated with MACRO 5.2, while pesticide losses via surface runoff and erosion are simulated using PRZM 4.51. Furthermore, GERDA includes the option of simulating vegetated filter strips with the model VFSMOD. Pesticide fate in surface water is calculated with the model STEPS. GERDA includes 12 climate scenarios, all 102 agriculturally relevant FOOTPRINT soil types (FSTs) for Germany and 49 different crops. The GERDA package includes 1296 spatial cumulative distribution functions (CDFs) of worst-case-ness corresponding to unique combinations of i) pathway: drainage or surface runoff, ii) Koc, iii) DT50 in soil, iv) application month, v) crop seasonality and vi) descriptor variable: median (annual PECsw,max) or median (annual area under the curve). The user first creates or selects a project and then chooses the desired cumulative area percentage of worst-case-ness. GERDA then determines internally the relevant 4-16 CDFs and reads off the soil/climate combinations corresponding to the selected percentage. The user can now launch the PRZM and MACRO simulations. Once the results of PRZM and MACRO are available, VFSMOD (if applicable) and STEPS can be run. For each STEPS simulation, the GERDA user can view a summary report containing an echo of the inputs and the registration-relevant outputs. Moreover, a 30-year hourly time series of PECsw/sed is stored which can be used for e.g. TKTD modelling. GERDA constitutes a major scientific improvement in comparison to FOCUSsw. The spatially probabilistic approach of GERDA enables informed regulatory decisions on a national level.

321

Losses of glyphosate and AMPA by runoff in a typical residential area: monitoring and a conceptual model

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Runoff from urban hard surfaces may be an important source of pesticides in urban streams. To investigate the behaviour of pesticide runoff from hard surfaces, a monitoring campaign was conducted in a typical Belgian residential area (9.5 ha) between 15 May and 10 August, 2013. The campaign yielded a relatively high-resolution dataset of rainfall, flow and concentrations of glyphosate and its major degradation product - aminomethylphosphonic acid (AMPA), as well as detailed information from surveys with residents on glyphosate application and the characteristics of the studied area. On the other hand, a new conceptual model will be proposed based on an extensive literature review, taking into account the spatial characteristics of the residential area. Here, we will first present the results from the monitoring campaign and then the model concept for simulating urban pesticide runoff. According to our surveys and measurements, less than 0,3% of applied glyphosate was detected at the outlet of storm drainage. Twelve rainfall events were analysed to generalize the glyphosate behaviours during runoff. Unsurprisingly, glyphosate losses during individual events are associated with a wide range of factors, including the application rate, the interval between application and rainfall event and the potential location of application. Meanwhile, a few factors are identified as non-determinant. For instance, the flow rate is found to have no correlation with the concentrations of glyphosate and AMPA. Yet, the wide range of influencing factors indicate monitoring alone is not sufficient to quantitatively explain the complex pesticide runoff processes. Therefore, a conceptual model will be proposed that considers hydrology-driven processes and non-hydrological processes. It will utilize the grid-cell as the spatial unit and user-defined time-step, therefore, taking proper spatial and temporal variability into account. This conceptual model will be incorporated into an existing dynamic distributed hydrological model. The resulting model, in its simple form, will be applicable in regulatory processes for tailor-made applications.

322

Feedback from ANSES on the use of FOCUS Surface Water tools for Southern zonal risk assessment

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Risk assessment of surface water contamination for the approval of active substances at European level is conducted with the European FOCUS (FOrum for the Coordination of pesticide fate models and their USe) exposure models. The objective of these tools is to determine whether a safe use for aquatic organisms can be identified in a European area. However, although initially established for European assessment, the FOCUS tools are now used in several European countries for aquatic risk assessment for preparations. This presentation gives feedbacks on ANSES experience on the use of FOCUS Surface Water exposure models in the framework of zonal dossiers. Risk assessment in France is performed considering all FOCUS Surface Water scenarios defined in Step 3, except D1 scenario. They cover the variety of agro-pedo-climatic conditions, and are used to identify acceptable or unacceptable risks for aquatic organisms, including mitigation options when appropriate. Key parameters of the active substances for describing the processes to be taken into account in the exposure models are usually considered to be the degradation rates and adsorption coefficients. However, some other input parameters have a very significant impact on the Predicted Environmental Concentrations in surface water (PECsw), as for example the Freundlich coefficient and the wash-off factor. In addition, parameters related to the application pattern are also very sensitive. Given that the FOCUS Surface Water models only consider a sixteen-month assessment period, the PECsw values are event driven and very related to the choice of the application window. This last point is considered as an important limit for the interpretation of the model outputs. Taking into account several years of repeated applications as done for PEC groundwater calculations would improve the robustness of aquatic risk assessment.

323

Proziris – a new tool to support pesticide registration at the national and zonal levels

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EFSA has recommended for national assessments to be undertaken to better evaluate the environmental risks of crop protection products: "... the FOCUS scenarios are limited to demonstrating safe use in a significant area in the EU… For national assessments, all crops and the entire potential use area must be considered." At the same time, EU Regulation 1107/2009 requires zonal risk assessments to be undertaken. Therefore, there is an urgent need for a tool able to perform leaching and surface water risk assessments for all agriculturally relevant areas of the EU. \nA multi-lingual web-based risk assessment platform has been developed to support pesticide registration at the national and zonal scales. The system connects a web interface with a supercomputer dedicated to pesticide fate modelling. The input interface allows users to specify the assessment details including i) geographical extent (country or zone), ii) crop, iii) pesticide application date(s) and rate(s), iv) physicochemical characteristics of active substance and metabolites, and v) eventual mitigation measures. Spatially-distributed pesticide fate modelling is undertaken using European-level datasets for soils SGDBE) and land cover / land use (CAPRI). The modelling is undertaken for 10 years using the PRZM 4.51 (surface runoff, erosion) and MACRO 5.2 (leaching, drainage, lateral subsurface flow) models based on the FOOTPRINT Soil Type approach. Spray drift input into surface water is calculated with the Ganzelmeier-Rautmann drift functions. Proziris is able to simulate mitigation measures for spray drift (drift-reducing nozzles, minimum distances) as well as for surface runoff and erosion (grassed buffer strips). Two options are available for buffer strips: a) reduction efficiencies as a function of width, b) dynamic simulation with VFSMOD. All pesticide and water fluxes into surface water bodies are fed into a recoded version of the STEPS model which calculates hourly PECsw, PECsed and PECporewater time series. The obtained PECsw/sed/pw and leaching time series are post-processed automatically to yield all relevant endpoints. The results of an assessment are provided to the user via the web output interface both in non-aggregated and in aggregated form (spatial CDFs, maps). Due to its spatially distributed and spatially probabilitistic approach, Proziris provides a much better accuracy and protectiveness than approaches with only a small number of scenarios like FOCUS.

Sorption and bioavailability of organic chemicals: mechanisms and applications in innovative remediation

324

Interaction of amines with natural soil studied with nitroxyl spin probes

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The environmental risk of organic xenobiotics released to soils is controlled by their transformation, sorption and binding processes. However, the molecular mechanisms of reversible and irreversible interactions of xenobiotics with soil constituents are only partly understood. We present a new approach of using stable electron spin probes to investigate the interactions of typical functional groups of xenobiotics with the molecular environment of natural soil. We used the nitroxyl spin labels TEMPO (2,2,6,6-Tetramethylpiperidin-1-oxyl), amino-TEMPO (4-amino-2,2,6,6-Tetramethylpiperidin-1-oxyl) and anilino-NO (2,5,5-Trimethyl-2-(3-aminophenyl)pyrrolidin-1-oxyl), which differ in their hydrophobicity and reactivity to soil organic matter. A significant broadening of the ESR-signals of anilino-NO incubated with luvisol soil indicates a strong restriction of the reorientational motion of the spin probe, which could not be observed with the other two spin probes. The same signals could be detected with the natural humic acid leonardite, which suggest a strong binding to soil humic substances, i.e. covalent binding. The unchanged signals of soil organic radicals rules out radical coupling and suggests a nucleophilic addition reaction as the covalent binding mechanism. Sequential desorption from incubated soil with increasing water volume revealed an almost exponential decrease of the percentage of all three spin probes remaining in the soil. The fractions of TEMPO and amino-TEMPO almost completely diminished with increasing volume of percolate, whereas those of aniline-NO approximated a stationary level of 6% remaining in soil. We conclude that large fractions of all three spin probes are weakly bound to soil with low binding energy, because they can be extracted by an excess of water. For anilino-NO, results indicate an additional fraction to be irreversibly bound to soil. This finding supports the conclusion from the ESR-spectroscopic analysis of the interaction of the spin probes. Our work clearly demonstrates for the first time the large benefit of using spin probes to investigate the interaction of xenobiotics with natural soil via their specific functional groups.

325

Including organic mixture's influence on dioxins and furans' bioavailability for toxic impact assessment in a life cycle context

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Polychlorodibenzo-p-dioxins and -furans (PCDD/Fs) are never produced on purpose but as undesired impurities of chlorinated compounds. The pentachlorophenol (PCP) pole-treating oil contains traces of PCDD/Fs and it has been proven that PCDD/Fs migrate deeper in the soil due to the oil's influence, which modifies the PCDD/Fs sorption behavior between soil and water in contaminated sites around PCP treated poles. In the generic context of Life Cycle Assessment (LCA), any interactions between co-contaminants are not included to characterize (eco)toxic impacts. Therefore the main objective of this study is to understand to what extent (eco)toxic impacts of PCDD/Fs would change by including the oil's influence on PCDD/Fs' fate in an LCA context. 2,3,7,8-tetrachlorodibenzodioxin (TCDD) was chosen as a proxy of the PCDD/F mixture. Based on literature, it is assumed that 1) the oil carries the PCDD/F in its environmental distribution in the same proportions, 2) the oil's degradation releases the PCDD/Fs, 3) they are considered as emitted from the compartment where the oil is degraded instead of being emitted in the initial emission compartment. The influenced TCDD's Characterization Factors (CF) are obtained from the influenced Fate Factors and by ignoring the oil's influences on PCDD/Fs' exposure and effects. The toxic impacts of the Canadian overall PCDD/F emissions in 2001 were then obtained. The approach led to a significant increase of potential toxic impacts when PCDD/Fs are emitted into soil only. The application of these CFs on the overall PCDD/F emissions in Canada in 2001 did not result in a high increase because the important change in CF did not overtake the small quantity of PCDD/Fs emitted into soil by poles compared to the overall emission. This work remains exploratory but represents a first attempt to integrate the contaminants' interactions in a LCA context and led to significant increases for TCDD's CFs for an emission into soil. However as the volatilization of PCDD/Fs through organic phases represents an important mechanism, it is recommended to lead studies on this topic to confirm or invalidate this approach.

326

Positioning activated carbon amendment technologies in a novel framework for sediment management

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Contaminated sediments can pose serious threats to human health and the environment by serving as a source of persistent toxic chemicals to humans or aquatic organisms. Amendment of contaminated sediments with strong sorbents like activated carbon (AC) is a rapidly developing management strategy. To date, much attention has been paid to the technical and ecological features and implications of sediment remediation with AC. The present work provides an evaluation of sediment remediation with AC as a sediment management option, in comparison with traditional remediation techniques in use, also covering the main factors related to their full-scale application. First, a summary of AC treatment technology based on recent reviews and the latest literature was provided. This included the effectiveness and ecological safety of the use of AC in natural systems. Then, a qualitative overview of (dis)advantages of current alternatives to remediate contaminated sediments was presented. Finally, this information was used to provide a novel framework for the supporting decisions concerning sediment remediation and re-use.

327

Changes in organic contaminant availability following biochar/activated carbon amendment for enhanced phytostabilisation of contaminated soils

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The potential role of biochars and activated carbons in remediating contaminated soils has been suggested many times before but a greater mechanistic understanding of the effects of amendment on contaminant availability and plant establishment is required before full scale field application. Previous work suggests plant establishment can be enhanced by amendment and contaminant availability can be reduced but the heterogeneous nature of different biochars means that this is not always the case. In order to further understand how biochar and activated carbon can enhance soil phytostabilisation and what their effect on PAH availability is, a 90 day outdoor pot trial with Lolium multiflorum (Italian ryegrasss) in coal tar contaminated soil was carried out. A total of 9 treatment and control scenarios were set up (n=5 per treatment) as follows: 1)soil control 2)soil + plant 3) soil + plant + NPK; treatments 4-6 and 7-9as 1-3 except with 1% maize biochar addition and 1% granular activated carbon addition respectively. On the hypothesis that the different treatments would significantly affect PAH uptake to plants and soil porewater PAH concentrations compared to controls, PAHs concentrations were assessed in the soil, soil porewater and plants across treatments. Polyoxymethylene (POM) samplers were used to assess porewater concentrations and predict plant uptake. Activated carbon amendment significantly reduced all PAH concentrations in porewater and plants while variable effects were observed for the biochar

amendment.

328
Investigating biochar and activated carbon effects on microbial community structures in petroleum hydrocarbon contaminated soils
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Organic compounds tend to persist in the environment and may become a serious cause for concern because of their detrimental effects to humans and the environment. It is therefore imperative to consistently develop effective approaches for the remediation of these compounds from the environment. A recently developed novel technique for the remediation of organic compounds is the use of sorbents to amend contaminated soils and sediments. Sorbents have the capacity to adsorb organic pollutants from the environment because of their porous nature. At the same time, sorption of organic pollutants reduces bioavailability of the pollutants to indigenous microorganisms present in soils and sediments. Because of these antagonistic effects, the net effects of sorbents on biodegradation of organic pollutants cannot be easily predicted. It is therefore necessary to gain a better understanding of the trade-offs which may or may not exist between sorption of organic compounds form the soil and biodegradation by soil microorganisms. In the current study, we investigated the effects of biochar and activated carbon on microbial community structures in volatile petroleum hydrocarbon contaminated soils. Previously uncontaminated gravelly sand was amended with 2% biochar (d.w.) and 2% activated carbon (d.w.) respectively. Soils (amended and unamended) were packed into three separate glass columns and a vial containing a mixture of 12 volatile petroleum hydrocarbons (VPHs) was connected to each column via a curved glass tube to serve as a non-aqueous phase liquid (NAPL) source. Soil pore respiration was monitored for a duration of 430 days after which glass columns were taken apart. Soil bacterial DNA was extracted from three sections of each column and the V4 and most of the V5 regions of 16S rRNA gene of DNA isolates were PCR amplified using barcoded 515f and 926r primers. Amplicons were sequenced on a pyrosequencing Roche 454 GS FLX + System and sequences analysed using the Quantitative Insights Into Microbial Ecology (QIIME) tool kit. The major bacterial taxa (relative abundance >1.5) identified include Deltaproteobacteria, Alphaproteobacteria, Clostridia and Bacilli all of which have been previously implicated in petroleum hydrocarbon degradation. Sorbent amendment also had a detectable effect on the relative abundances of bacterial communities between the treatments ($p< 0.05$).
Keywords: Bioavailabilty, VPHs, pyrosequencing, biodegradation

329
Tools for the evaluation of remediation process in Microbial Electroremediating Cell (MERC).
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We presents the techniques using to evaluate Microbial Electroremediating Cell (MERC), a novel tool recently propose by this authors, based on Microbial Fuel Cell, for in situ soil remediation. Our results support the concept of electrogenesis-stimulated bioremediation, which consist in stimulating the microbial oxidation of the pollutant by supplying an anode as extra electron acceptor. For evaluating MERC, we have design a special set up which allow to assay polluted soil samples under different conditions to evaluate the relevance of biological process and physicochemical process (pollutant sorption in soil and electrode materials; electrochemistry mediated transformations). Using differents techniques we confirm MERC as an interesting and efficient methodology which offers the possibility of a) monitoring the in situ pollutant biodegradation using environmentally-friendly tools, and b) stimulate native populations, overcoming the availability in electron acceptor that typically limits biodegradation of organic pollutants, without a sever modification of the soil.

Advancements in life cycle impact assessment and footprint method development (III)

330
Chemical footprint assessment: presentation of method and application to a case study involving different spatial scales
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Expressing Life Cycle Impact Assessment (LCIA) results as footprints is gaining increasing attention by scientific and political communities alike. Footprint assessments have the potential to improve our ability to communicate environmental impacts to stakeholders since footprints are expressed in units more intuitive than those of traditional LCIA impact categories. Furthermore some footprint methods, such as the ecological footprint, can be compared with a carrying capacity, which qualifies them as indicators relevant to environmental sustainability assessment. Footprint methods related to land use, water use and carbon emissions are well established, but as of now, no operational chemical footprint (ChF) indicator exists. We present a newly developed ChF method for quantifying the combined ecotoxicological impact in freshwater from emissions within a territory. In this context a ChF is defined as "the occupation of a (theoretical) fresh water volume needed to dilute a chemical emission to the point where it causes no damage to ecosystems in the volume during its presence". $HC5_{NOEC}$, the concentration at which a maximum 5% of modeled ecosystem species are affected, is used as a safe reference concentration. This allows for the conversion of the USEtox output [PAF.m^3.day] into a ChF [km^3]. Results can be compared to the availability of surface freshwater at the relevant spatial scale. We tested the feasibility of the method by applying it to an inventory of the chemical emissions within Europe in 2004. We found that the ChF of several large European cities exceeded the carrying capacity of surrounding freshwater bodies by more than 10 times, although the carrying capacity of all European surface freshwaters on average was close to non-exceedance. This observation illustrates the inhomogenous distribution of surface freshwater and chemical emissions within the European continent and the importance of focusing territorial ChF assessments on cities and their surrounding freshwater bodies. This method of calculating ChF for freshwaters has the benefit of being easily communicated to decision makers and furthermore, is relevant for environmental sustainability assessment because it directly relates to the environment's carrying capacity. The weakest link in the assessment is knowledge of emission inventories at the spatial scale of cities to small regions.

331
Great ideas – greater difficulties in their modelling: when LCIA hits a dead-end. A tale of ecotoxic struggle, with a silver lining.
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Existing aquatic ecotoxicity effect models employed in life cycle impact assessment (LCIA) inadequately address the need to characterise complex chemical mixtures, such as industrial effluents, since the former have been developed explicitly for emissions of single chemicals. Effluent emissions form part of the lifecyle of numerous products, so there is great motivation to find meaningful ways of characterising the effect these effluents have as a whole, avoiding the need to make rough estimations based on approximations of the comprising individual chemicals. One such meaningful way of quantifying the relative ecotoxic potential of industrial effluents would be via organic sum-parameters, such as TOC, COD, or BOD. These measures of bulk organic content are widespread and easy to measure, so characterising their ecotoxic effect would equate to bypassing the need to estimate their composition. Based on whole effluent toxicity (WET) test results, the idea is to roughly 'split' the effluent into two parts, the organic and the inorganic fraction, and then deduct from the toxicity of the whole effluent the toxicity of the inorganic fraction. If the mixture toxicity principle of concentration addition is valid for these two *groups* of chemicals, then we can expect the remaining toxicity to be attributable to the organics, and we can assign this to a measure of organic content, e.g. TOC. We now have a way of estimating the measure of ecotoxicity for TOC, which will make it comparable to individual chemicals, namely its EC50. With results for several aquatic species, we can then calculate an effect factor for TOC (EF_{TOC}). The elegance of this idea lies in its overall simplicity in the way we think of effluents, and in the way it promises to gives us a means of characterising the bulk of the effluents. However, this overall simple framework is actually made up of difficult and data-intensive steps, which require several assumptions to be made. In this work we present these steps in detail, and our effort to extract something useful for LCIA from the results. LCIA method development often hits a dead-end, despite great ideas and even greater efforts, but there is a silver lining, because if not in LCIA, the results obtained can be applied to other fields.

332
Development of characterization factors for metals in coastal seawater
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Metal ecotoxicity Characterization Factors (CFs) in coastal seawater were not developed in USEtox for Life Cycle Impact Assessment (LCIA), due to lack of appropriate ecotoxicity data and inadequate consideration of metal bioavailability in seawater. Taking into account the speciation behavior of metals in seawater and using effect data exclusively for marine organisms, two sets of spatially differentiated CFs were developed for the metals Cd, Co, Cu(II), Ni, Pb and Zn in coastal seawater. One set of CFs ($CF_{sw\text{-}sw}$) addresses the direct metal emission to coastal seawater while the other set ($CF_{fw\text{-}sw}$) represents the ecotoxicity potential of metals in coastal seawater caused by metal emission to freshwater followed by transport to the seawater compartment, taking into account the fate of metal in freshwater and in the estuary. CF is the product of three factors: Fate Factor (FF), Bioavailability Factor (BF), and Effect Factor (EF). The multimedia fate model of USEtox was used to calculate the FF. WHAM 7.0 was used to model metal speciation underlying the BF. Free Ion Activity Model (FIAM) was used to model EF. The results showed that for a given metal, FFin seawater was higher than in freshwater, due to longer residence time of the water in the coastal seawater than in freshwater. The difference between FF in seawater and freshwater was smaller since the difference in water residence time was partially neutralized by metal removal in estuaries. Metal BFs in seawater were similar or slightly higher than in freshwater due to the lower DOC and SPM concentration in seawater. For most metals, EFs were lower in seawater than freshwater, due to lower sensitivity of seawater biota to metals. As a general observation, $CF_{fw\text{-}sw}$ was lower than $CF_{sw\text{-}sw}$ due to metal removal in freshwater and estuary, but the difference was modest for most metals. For Pb , seawater CF were up to 1-4 orders of magnitude higher than freshwater CF. But for the other metals, seawater CFs were similar to freshwater CFs, indicating that the higher FF and BF in seawater were largely counterweighed by the lower EF for these metals. The variation of CFs in different coastal seawaters were up to ca. 2-3 orders of magnitude for one metal, indicating the importance of using spatial differentiated CFs. Compared with USES-LCA default $CF_{sw\text{-}sw}$, the new $CF_{sw\text{-}sw}$ were at least 3 orders of magnitude lower for all metals except Pb, of which USES-LCA $CF_{sw\text{-}sw}$ fall within the range of this study. This implied that for some metals, ecotoxicity CFs in coastal seawater might be overestimated in previous LCIA methods.

333
Confronting Health Effects of Particulate Matter in LCIA
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Ambient particulate matter (PM) is one of the most important environmental stressors contributing to the global human disease burden. However, there a lack of guidance on how to include health effects from PM exposure in the health footprint for life cycle impact assessment (LCIA). A task force was initiated to build a PM health impact framework and factors based on scientific consensus. Existing literature was reviewed and expert input was collected and discussed in an initial Guidance Workshop. Key scientific questions and challenges for quantifying health effects from PM exposure have been discussed and initial guidance and recommendations for the upcoming impact quantification process were developed. Preliminary recommendations address the general assessment framework, aspects to determine intake fractions (iF), and aspects to determine exposure-response factors (ERF) along with disease severity: These recommendations include: (1) the 2011 framework proposed by Humbert et al. (doi:10.1021/es103563z) provides an assessment starting point; (2) iF can be used as exposure metric with breathing rate linking ambient concentration and intake; (3) disability-adjusted life years without age-weighting and discounting can be used as a health metric; (4) population archetypes can account for aspects influencing intake fractions; (5) spatially-differentiated iF should be established for all archetypes with geographic differentiation further discussed; (6) emission-weighted iF are needed in all cases where emission and/or exposure conditions are unclear; (7) the 2010 Global Burden of Disease Study provides a useful starting point for calculating health effects; (8) compared to all-cause mortality, cause-specific mortality can provide a more informative basis an LCIA metric but age- and cause-specific disability weights need further analyses; (9) the need remains to discuss whether and how to consistently integrate non-linear exposure-response into LCIA; and (10) $PM_{2.5}$can be used as indicator of the health risk associated with PM inhalation exposure. There is not sufficient evidence-based justification to differentiate between different primary/secondary PM sources or between different particle sizes regarding toxicity. Our study constitutes a first step towards arriving at recommendations for how to account for health effects of emissions of primary PM and secondary PM precursors in LCIA. However, a range of inconclusive aspects requires further analysis.

334
Using machine learning for human toxicity and freshwater ecotoxicity characterization of chemical emissions
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Toxicity characterization of chemical emissions is a complex task which usually proceeds via multimedia models attached to models of dose–response relationships to assess the effects on targets. Different models and approaches are available, but all require a vast amount of data on the properties of the chemical compounds being assessed, which are hard to collect or hardly available (especially for less common or newly developed chemicals). An example of such models is USEtox™, a consensual model for the characterization of human toxicity and freshwater eco-toxicity. The final aim of this work is building a data-driven model for chemical characterization from a limited amount of substance-specific data, complementary (and not alternative) to the existing assessment models. By focusing on Usetox™, and more specifically on the modelling of the fate factor (FF) from continental urban air to urban air ($FF_{airU,airU}$), for which data are available, this work makes a step ahead in that direction by pursuing two main objectives: 1) performing for the first time an extensive exploratory data analysis (EDA) of the input space containing substance-specific properties at the aim of detecting particular patterns in the data manifold; 2) exploring the modelling efficiency (for predicting toxicity) of a set of algorithms based on linear partial least squares (PLS) regression and on non-linear approaches: kernel PLS (KPLS), adaptive general regression neural networks (GRNN) and random forests (RF). The database available in USEtox™ wasused in this study. For the sake of simplicity, only the organic compounds have been taken into account here, to facilitate the development and testing of the approach pursued here, while not affecting the consistency of the outcomes and their applicability to the other compounds. Four main explanatory variables (degradation rate in air, degradation rate in water, Henry law coefficient at 25°C and partitioning coefficient between octanol and water) were identified in the input space. They are, therefore, the most important variables which have to be assessed with a high degree of accuracy. Current research efforts are addressing other parts of the model affected by important data gaps, e.g. to the calculation of human health effect factors. The presentation will focus on the results from non-linear algorithms and on the added value of the data-driven model for the LCA community as a whole and the toxicity assessment practice in particular.

Monitoring the efficiency of risk mitigation measure protecting the environment from pesticide exposure and effects

335
Feedback from MAgPIE workshop - Environmental risk mitigation measures in risk assessment and management for P
V. Poulsen, ANSES; A. Alix, Dow Agrosciences / Risk Management
A European workshop under the auspices of SETAC and European Commision was organised in order to provide European regulatory authorities a toolbox of risk mitigation measures designed for the use of Plant Protection Products for agricultural purposes. During the two workshops (one in Rome and one in Madrid) and due to their work in-between, stakeholders provided, for groundwater, surface water, in-field terrestrial and soil organisms, and off-field terrestrial organisms: - A summary of existing risk mitigation measures used in the different countries for regulatory purposes, - An analysis of these risk mitigation measures according to: their effciciency, their potential use in risk asessment, their practicallity of implementation for farmers, their practicallity of implementation from a regulatory point of view, - A collection of voluntary initiatives and stewardship programmes, - Feedback on experiences in risk management, - Identification of needs for adaptation/proposal of new S phrases, - Identification of needs for further developpements. For each compartment of the environment, participants prepared a common risk mitigation toolbox in which member states could chose the tools being most appropriate for their specific legal and agro environmental conditions whilst ensuring the same level of protection as in the rest of Europe. Proceedings are in preparation and are intended to be finalized in 2014. They will reflect the state of the art of environmental risk mtigation measures for plant protection products, and provide analyses of these measures according to the discussions among all participants. They will therefore provide risk managers and risk assessors with an efficient toolbox. A website will contain all suitable information to be shared by stakeholders, and to be communicated to farmers in order to help them implementing the most relevant measures at a local scale.

336
Groundwater protection measures for use of Plant Protection Products
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The response of groundwater residues to the introduction of mitigation measures can extend to decades, therefore a combination of fate and behaviour data, local hydrogeological conditions and modelling is commonly required to help design appropriate best management practices for Plant Protection Products (PPP). Mitigation measures can be categorised according to the process or behaviour they are designed to address for example: the field setting (intrinsic soil properties and climatic conditions), the built environment (presence of drinking water abstraction wells), farmer practice and choices and finally the chemical characteristics of the PPP. Relevant mitigation measures may include the exclusion of vulnerable zones with shallow groundwater and porous soils, control of irrigation and using the most appropriate PPP application rate, timing and technique. Currently there is no model routinely used in the PPP regulatory process that describes the potential for residues to be found in groundwater below one metre depth. However real-world monitoring has clearly shown that a combination of important environmental processes, actual farmer use rates and an assessment endpoint in shallow groundwater (The number of mitigation options available to minimise the potential for PPPs to be transported

to groundwater is limited. Additionally a long timeframe is unavoidable for the evaluation of trends indicating satisfactory outcomes from groundwater related best practices. There is, therefore, a pressing need for agreed and validated methodologies to model the effect of such mitigation options within the regulatory process.

337
Monitoring long-term trends of pesticides in surface waters
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Environmental monitoring of pesticides in Sweden started during the mid-80s as short term, research based investigations of possible occurrence of pesticides in streams and rivers. However, during the last decade the monitoring program has expanded substantially. Much effort was directed towards including relevant parameters to enhance interpretation possibilities. Parameters collected include detailed pesticide use data within the catchments, continuous flow measurements, precipitation and soil data. Water sampling is performed continuously with automatic samplers at the catchment outlet. Information on pesticide use enables us to adapt the analytical programme, aiming at including ca 90% of the total volume applied in the catchment area. Results from the programme are e.g. used to assess the success of national legislation and risk management programmes. Some overall results will be presented, including results from one catchment reflecting long-term (20 y) changes in pesticide concentrations due to increased awareness amongst the farmers. Detected concentrations will be compared with the EU EQS and Swedish threshold values for pesticides. This comparison is performed using a Pesticide Toxicity Index (PTI). Results from pesticide monitoring programmes generate information that help researchers, industry and regulators to better understand the pesticide-environment relationship. Results generated can also be used to ensure that the environmental behaviour of pesticides is in accordance with regulatory intentions and applications done with the use of best management practices. This forms the basis for better communication of appropriate practices for minimising possible negative impacts from pesticide use on the environment.

338
Risk mitigation of pesticides
M. Streloke, BVL / Plant Protection Products

339
Risk management for pollinators: regulatory context, overview of risk management tools and perspectives
A. Alix, Dow Agrosciences / Risk Management; C. Garrido, IBACON; M. Miles, Bayer CropScience UK / Analytical Chemistry and Environmental Sciences; E. Johansen, Washington Dept of Agriculture; B. golla, Institute for Strategies and Technology Assessment
Risk mitigation measures dedicated to pollinators are of increasing importance for environmental protection in the area of the use of pesticides in crop protection. The question raises multiple exchanges between European authorities, and many initiatives have been undertaken in order to develop, implement and account for risk mitigation measures in the risk assessment procedures. The Organisation for Economical Co-Operation and Development (OECD) has undertaken surveys aiming at collecting risk mitigation practices in OECD countries. In May and November 2013, a European workshop under the auspices of SETAC and European Commission was organised in order to provide European regulatory authorities with a toolbox of risk mitigation measures designed for the use of Plant Protection Products for agricultural purposes. This presentation will illustrate the outcome of the work undertaken by these organisations in the inventory and review of the risk mitigation measures developed and implemented to protect managed and wild bees in agricultural landscape.

340
Plant protection products in surface water: how inputs can be reduced on-farm
O. Daniel, Agroscope ACW; L. Buehler, Agroscope
Agriculture aims at keeping the input of plant protection products (PPP) into surface water to a minimum. Measures to reach this goal are a sophisticated authorisation process, restraints bound to the authorisation such as distances from edge of field to surface water, restraints from agri-environmental schemes such as a 6 m buffer strip, periodic control of the spraying equipment and training of the farmers. Nevertheless, PPP are found in surface water at both, concentrations below and above regulatory thresholds. Agricultural structures, soil and topographical properties vary in Switzerland within a very small scale. Therefore, it is believed that a further minimisation can be achieved by on-farm and intra-parcel measures. We present a categorisation tool which considers erosion potential, soil properties and connectivity to surface water. Each parcel of a farm is categorised with respect of being a potential PPP source, contributing to the transport of PPP (run-off, infiltration) and the connectivity (surface: run-off, sub-surface: drainage) to surface water. Connectivity is affected by physical barriers, the type of neighbourhood parcel, drainages in plain areas, drainages in sinks, gullies in streets and vegetated buffers. Information on the parcels was taken from various GIS maps (e.g. erosion potential), local maps (drainage, parcel plan) and orthofotos. This information was cross-checked and supplemented with information gathered from an interview with farmers and a field inspection. A pilot study with three farms came to the result that: a) hot spots for PPP inputs into surface water can be localised, and the potential of PPP input into surface water can be categorised. b) identification and categorisation allow to prioritise and allocate suitable measures for a reduction of PPP inputs in surface water. c) it has to be distinguished between potential and actual inputs into surface water. In the farms studied several measures to reduce PPP transport, such as an adequate crop rotation, direct drilling and ecological compensation areas, have been implemented by the farmers already.

Environmental OMICs: high-throughput strategies to decipher mechanism of response to stressors (I)

341
Mechanisms of response to Cu and Ag materials: gene expression profile in Enchytraeus crypticus
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High-throughput gene expression tools help understanding the mechanisms of toxic-mediated responses. Further, to establish the link between alterations in critical macromolecules (e.g. genes, proteins, metabolites) and their possible biological implications at higher levels (survival, reproduction) represents one of the major milestones. Such data can be integrated e.g. via the Adverse Outcome Pathways (AOPs) approach, improving risk assessment. The aim of the present study is to investigate the mechanisms of toxicity for Cu (copper) and Ag (silver) materials using the high-throughput tool for the soil worm *Enchytraeus crypticus* (Oligochaeta), a 4x44K custom Agilent microarray. Testing was done based on reproduction effect concentrations (EC_{20}, EC_{50}) using 3 and 7 days of exposure. Results indicated specific mechanisms of response for the different materials tested. Cu-salt exposure affected mechanisms related with calcium homeostasis and activated the chemosensory system of the enchytraieds. Energetic metabolism was affected differently depending on the copper forms. For Ag, results showed that one of the materials caused a more differentiated transcriptomic profile than the others. Commonly across all Ag forms were the effects on cell cycle control associated with impairment of DNA repair mechanism. The study of gene expression pointed at differences in gene responses to the various Cu and Ag forms tested, information that is notoriously absent in experiments focussing on the standard effect endpoints survival/reproduction. The analysis of Adverse Outcome Pathways (AOP) was a promising approach to integrate effects from various levels and provide input for risk assessors. **Keywords:** High-throughput, mechanisms of response, (nano)materials, soil;

342
Arabidopsis thaliana defense mechanism to Ag- and TiO2-nanoparticles, and carbon nanotubes exposure from transcriptomics analysis
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There are increasing concerns about the putative impact of released nanoparticles (NPs) on the environment and their toxicity for living organisms. Since plants represent by far the largest interface between environment and biosphere, they will be the first barrier for nanoimpact. The study of detoxification and plant defense mechanisms displayed upon NPs exposure might be of major relevance and transcriptomics could provide information of the regulatory network involved in this process. Here we used the model plant *Arabidopsis thaliana* to study the changes of plant transcriptome associated to NPs exposure. By using Agilent expression microarrays we performed transcript profiling on more than 30k genes in the plant genome. We compared the expression patterns associated to NP exposure with those produced by biotic (infection with biotrophic or necrotropic pathogens) or abiotic (salt, draught or mechanical) stress. These stressors are naturally produced as a consequence of environmental or biological changes and plants manage to survive their deleterious effects by activating the expression of an arsenal of defense-related genes. We found that exposure to NPs caused mild changes in gene expression when compared to the mayor effects of the biotic or abiotic stress tested here. Principal Component Analysis indicates that the main component in transcriptome changes is related to necrotrophe fungal infection, followed by draught and other abiotic stress, whereas exposure to Ag and TiO2 NPs (10, 20, 40 or 80 nm) or carbon nanotubes cluster together in the last position of PCA. Significance Analysis of Microarrays defined a set of 278 gene-probes for which differential expression is verified upon exposure to all the types of NPs tested

here. Many of them are also key regulators of the pathways that are triggered during plant immune and stress responses.

343
Adverse outcome signalling pathways of dioxin-like compounds in Danio rerio
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Zebrafish embryos constitute a convenient model for studying effects of environmental stressors on development. Among these stressors, dioxin-like pollutants are especially relevant because of their ubiquity among environmental compartments and their known toxic effects (oxidative stress, genotoxicity, cardiotoxicity, and others) in many animal species, particularly in vertebrates. The study performed allowed to detect the transcriptional responses of *Danio rerio* embryos at sublethal concentrations of three compounds with recognized dioxin-like activity, two PAHs (Benzo[k]fluoranthene, and Benzo[a]pyrene) and the natural compound β-Naphtoflavone. A total of 2201 unique genes (out of 15,883) appeared as differentially affected ($p < 0.001$) by at least one of the treatments. Cluster analysis separated data from β-Naphtoflavone treated samples from the PAH treated ones. A defined cluster of genes showed a common pattern of variation for the three treatments, including an enhanced expression of the cytochrome P450 superfamily and other stress- related genes, corresponding to the well-known activation of the regulatory AhR by dioxin-like compounds. Another interesting group of genes was those whose transcripts were only affected upon a long (5 days) longer exposure to Benzo[a]pyrene, a known mutagen. This group included many mitochondrial genes and other ones related to apoptosis or to cell death. We consider that these data suggest a novel mechanism of embryonic toxicity for B[a]Pyr, which compromises the cellular energy process and that may be related with other well known effects of B[a]Pyr, like cardiotoxicity or edemas. The analysis performed here may contribute to distinguish between different modes of action for pollutants that may share some targets in the cell (in this case, the aryl hydrocarbon receptor) but that, at the same time, differ in their toxic effects at the systemic level. Zebrafish embryos thus demonstrate their suitability for predicting the potential for developmental toxicity of environmental air/water samples. Our goal is to use this transcriptomic data to evaluate the toxicity of PAH-rich environmental samples from different origins (atmospheric, aquatic) and composition.

344
Using transcriptomics to evaluate the impacts of paper mill effluent, androgens, and progestins on a potential bioindicator organism, the eastern mosquitofish (Gambusia holbrooki)
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OMICs technologies have the potential to enhance the usability of bioindicator organisms in the field, with molecular changes providing insights into a toxicant's mode of action. Recently, progress has been made in developing a custom microarray for the eastern mosquitofish (*Gambusia holbrooki),* a potential bioindicator organism for endocrine disruptors. This species has a wide freshwater distribution and steroid-driven secondary sexual characteristics, and this non-model organism is now a viable bioindicator candidate. In this study, we describe the use of this custom microarray to evaluate the impacts of paper mill effluent and progestin exposures on *G. holbrooki*. Fish were collected from the Fenholloway River, a paper mill impacted site which has historically contained masculinized female mosquitofish, in addition to a reference site along the Econfina River. Liver samples of four female *G. holbrooki* were analyzed by microarrays and these data were compared to microarray data of female mosquitofish exposed to a potent androgen in the lab. Microarray analysis revealed a set of 62 genes that were similarly expressed between the paper mill and androgen exposed mosquitofish, with commonalities in biological pathways also demonstrating a similarity between the impacts of androgens and paper mill effluents. Due to the high presence of natural progesterone at the paper mill-impacted site, additional microarray analysis was conducted to determine the modes of action of synthetic progesterones on mosquitofish. One such chemical, levonorgestrel, also causes masculinization in fish, so we evaluated the ability of this chemical to elicit androgenic impacts on a transcriptional level. Female *G. holbrooki* exposed to 100 ng/L of levonorgestrel had biological processes that were modulated in a pattern similar to androgen-exposed *G. holbrooki,* indicating that both androgens and progestins may be working to disrupt specific metabolic processes. These data are supportive of an androgenic exposure at the paper mill impacted site and indicate that there are overlaps between the impacts of androgen and progestin exposures. This study was a successful implementation of microarray technologies for evaluating the mode of action of chemical exposures occurring in the field at a paper mill impacted site as well as for providing new insights on the biological effects of progestin exposure.

345
Gene expression meta-analysis reveals a gene set that acts in a dose dependent manner suitable for effect based screening
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The incorporation of genomic techniques into environmental risk assessment is a slow and challenging process since it is difficult to separate effects caused by chemicals from effects caused by confounding factors. Gene expression analyses tend to give very large, complicated datasets that will not supply the straightforward answers sought by ERA. It is therefore thought that smaller sets of genes that act as biomarkers and asses effects rather than compounds may be more suitable. To assess effect responses we performed a meta-analysis on 13 different gene expression studies in the springtail Folsomia candida that all assess different chemicals at EC10 and EC50 and compared these to the control group. 135 genes were significant over all exposures in both EC10 and EC50 as compared to the control. Gene Ontology analysis on these genes revealed many significant GO terms in detoxification . In a case study with increasing concentrations of phenanthrene and cadmium, 30 genes showed a significant monotonic increase or decrease of expression with increasing concentrations of phenanthrene and 9 genes showed this significant effect with cadmium. We present a set of 38 genes that were significant over all exposures and showed a significant monotonic response to either phenanthrene or cadmium.

346
Linking toxicity and adaptive response pathways across the transcriptome, proteome and phenotype of Chlamydomonas reinhardtii exposed to silver
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Understanding mechanistic and cellular events underlying a toxicological outcome allows the prediction of impact of environmental stressors to organisms living in different habitats. A systems based approach aids in characterizing molecular events, and thereby the cellular pathways, that have been perturbed. However, mapping just adverse outcomes of a toxicant falls short of describing the stress or adaptive response that is mounted to maintain homeostasis on perturbations and may confer resistance to the toxic insult. Silver is a potential threat to aquatic organisms due to the increasing use of silver-based nanomaterials, which release free silver ions. The effects of silver were investigated at the transcriptome, proteome and cellular level of *Chlamydomonas reinhardtii*. The cells instigate a fast transcriptome and proteome response, including perturbations in copper transport system and detoxification mechanisms. Silver causes an initial toxic insult, which leads to a plummeting of ATP and photosynthesis and damage due to oxidative stress. In response, the cells mount a defense reaction to combat oxidative stress and to eliminate silver via efflux transporters. From the analysis of the perturbations of the cell's functions, we derived a detailed mechanistic understanding of temporal dynamics of toxicity and adaptive response pathways for *C. reinhardtii* exposed to silver.

Soil Biodiversity and Ecotoxicology (I)

347
Earthworms responses to pesticide stress: adaptation strategies and soil implications
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The repeated application of pesticides in modern agriculture has led to chronic contaminations of cropped soils. The persistence and toxicity of several of these chemicals raises concern about adverse effects on non-target organisms of the soil biota such as earthworms (Lumbricidae). Although earthworm populations numbers are reduced in intensively cultivated soils, the persistence of certain species such as *Aporrectodea* spp in cropped soils suggests that adaptation processes to residual pesticide pollution can occur. We selected five fields exhibiting different chemical and farming histories within a joint agricultural area (acidic loamy-clay soils) and ranging from conventional cultivated to organic pasture. We investigated whether earthworm (*A. caliginosa and A. chlorotica)* responses to chemical exposure depend on the pesticide pressure in the soil they originate from by comparing pre-exposed populations (conventional farming) and naïve ones (organic farming). We thus analysed earthworm responses to environmentally realistic concentrations of two model pollutants in terms of

detoxication enzymes, metabolome, and burrowing behaviour. Soil pesticide analysis revealed 9 molecules in low levels in the three contaminated fields, amongst them atrazine, despite its ban in France since 2003. Basal activities of soluble glutathione-S-transferases and catalase were higher in *A. caliginosa* directly sampled from the fields with increasing pesticide pressure in the soil. Pesticide stress was reflected in the energy reserves of *A. chlorotica* in the conventional fields. Upon exposure to an environmentally realistic concentration of the fungicide epoxiconazole, GST activity was significantly increased in the pre-exposed populations (i.e deriving from conventional farming) after 7 and 28 days, and this was associated with changes in their metabolome after 28 days. A clear cut-off of metabolic profiles was observed only in the pre-exposed population between fungicide-exposed earthworms and the control group, with an increase in several of the metabolites and in particular amino-acids. There was also a higher burrowing activity leading to faster disappearance of the pesticide in the soil containing the pre-exposed earthworms. By contrast, these responses were not observed with the earthworms from the organic field. These findings demonstrate a physiological adaptation to pesticides in pre-exposed earthworms having possible consequences for the ecosystem level in terms of soil bioturbation.

348

Impact of Biosolids on Structural Endpoints of Soil Fauna

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Biosolids, i.e., sewage sludge after appropriate treatment such as anaerobic digestion, is applied to agricultural land worldwide. Due to its high content of complex and readily biodegradable organic matter as well as its content of nutrients such as nitrogen and phosphorus, biosolids improve soil fertility and quality similar to an amendment with other organic fertilizers. Yet, there are not only beneficial effects but also concerns about negative effects of biosolids amendment that relate, among others, to the contamination of groundwater and soil with biosolids-associated metals and organic pollutants. The present study investigated short-term and long-term effects of biosolids amendment on the soil fauna groups of nematodes, enchytraeids and earthworms at two field sites in Canada. The soil parameters organic matter and soil texture differed significantly between the control and the biosolids-treated parts at both field sites. But the difference was apparently not correlated with the biosolids treatment, which allowed assessing the biosolids effect independently from the influence of soil texture and organic matter content. The total abundance of the soil fauna groups nematodes, enchytraeids, and earthworms did not indicate an adverse long-term impact of biosolids application, but clearly illustrated the expected abundance-enhancing short-term effect resulting from the addition of organic material. Yet, the species composition within these groups indicated differences between biosolids-amended and not-amended parts of the fields in the long-term that appeared not to be dependent on other soil parameters such as organic matter content and soil texture. This study may thereby allow identification of indicator species for biosolids amendment or, more general, organic matter amendment in the past.

349

A new approach for the evaluation of soil toxicity due to atmospheric fall-out

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In the last decades the presence of toxic chemicals in the air has been considered a major environmental problem, both at ecosystem level and for human health. Air quality is often a major determinant of the level of the toxic chemicals that accumulate into the top soil. Our aim was to investigate the effects of the air pollutant fall-out on the soil. Field experiments were carried out using passive samplers consisting of plastic containers with a microperforated bottom containing OECD standard soil. The containers were maintained for three months in areas with different levels of anthropogenic pressures (Site 1: uncontaminated rural area; Site 2: botanic garden; Sites 3-5: areas close to a road with heavy traffic and industrial pollution). Soils were analysed for chemical content and toxicity. For this purpose, we utilised the social amoeba *Dictyostelium discoideum* and the earthworm *Eisenia andrei*, which are suitable for testing elutriate and soil toxicity respectively. Different endpoints such as mortality and reproduction as well as a battery of biomarkers (e.g., lysosomal alterations, ROS production, mitochondrial functionality and genotoxic effects) were studied. In soils from sites sampled close to a road with heavy traffic, high concentrations of toxic chemicals such as PAHs and heavy metals were detected. The biological results indicated that the soil elutriates have no effects on the different parameters evaluated in *D. discoideum*. The assessment of OECD soil toxicity by the earthworm *E. andrei* indicated in Sites 3-5 minimal effects at the organism level; however, more severe changes at the molecular/cellular level were found. In this context, a strong decrease in lysosomal membrane stability and mitochondrial functionality was observed in coelomocytes of exposed worms. Furthermore, a significant increase of oxidative stress as well as of lysosome/cytoplasm ratio in the chloragogenous tissue was also measured. Of relevance are the genotoxicity data; a significant increase in the level of oxidative DNA damage and micronuclei frequency was demonstrated. Worms incubated in the soil from Site 2, maintained in the botanic garden, showed only minimal increase of lipofuscin content. The role of PAHs contamination in causing toxic and genotoxic effects was confirmed by immunohistochemical results showing intense PAH-related fluorescence staining in tissues of worms exposed to soils placed in Sites 3-5.

350

Evaluation of the risk for soil organisms under real conditions

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It has been questioned in the last years if the risk assessment of Plant Protection Products (PPP) in soil addresses properly the relationship between pesticide exposure and effects on soil organisms. Thus, it is necessary to develop a new approach especially regarding the estimation of environmental concentrations within the soil profile. Two different outdoor studies and one indoor study were conducted by means of Terrestrial Model Ecosystems to measure and analyse the fate and effects on soil organisms (oribatid mites, collembolans, earthworms) of three different PPP with different physico-chemical properties (Lindane, Imidacloprid, Carbendazim) over time. Different soil layers (0-1 cm for pesticides only, 0-2,5 cm, 2,5-5 cm, 5-10 cm, 10-20 cm), were separately analysed and summarising results are shown for different soil arthropods (collembolans, oribatid mites, earthworms) and pesticides. The results of the presented study led to concrete recommendations that are given for risk assessment concerning exposure and risk of soil organisms under realistic conditions.

351

Impact of no-till versus conventional maize plantation in soil mesofauna: a TME experiment

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The use of different strategies in crop cultures affects soil mesofauna differently [2], which may interfere indirectly with soil productivity. No-till cropping constitutes a way of growing crops without disturbing the soil through tillage, preserving the soil structure (increasing water retention in the soil) and soil organic matter (nutrients) and decreasing soil erosion. On the other hand, conventional cropping includes soil tillage to remove weeds, shaping the soil into rows for crop plants and furrows for irrigation, which contributes to organic matter losses, degradation of soil aggregates and disruption of soil communities. Understanding the impact of these different cropping systems (no-till *vs.* conventional) in soil mesofauna and investigating the ability of soil communities to recover may provide important information to optimize cropping strategies to protect soil biodiversity and, consequently, preserve soil productivity. Aiming to fill this gap of knowledge, a terrestrial model ecosystem (TME) experiment was performed over two months using soil cores collected in a pasture area. The test treatments were: undisturbed soil with maize (control), mobilized soil with maize (conventional tillage); mobilized soil with maize and insecticide (conventional tillage with insecticide); undisturbed soil with herbicide and maize (no till); undisturbed soil with herbicide and insecticide (no till with insecticide). Soil samples were collected before soil mobilization (T0), after mobilization or herbicide application (T1) and 4 (T2) and 8 weeks (T3) after maize planting or maize planting and insecticide application. In each TME soil sample from 0-5 and 5-10 cm top layer were collected and collembolan and mite communities were identified and enchytraeids abundance was determined. Results suggest that soil mobilization (conventional cropping) affects collembolan and mite communities, making them more susceotible to insecticide application. Although in no-till cropping the insecticide application showed toxic effects especially to mite communities (in 0 to 5 cm top layer), there was a recovery of these communities at the last sampling date (after 8 weeks insecticide application; T3). In general, mite communities were more affected at surface soil layers (0 to 5 cm top layer) than collembolan communities. The

enchytraeids (at least in terms of abundance) showed low sensitivity towards the different cropping systems.

352

Species composition of a soil invertebrate multi-species test system determines the level of eco-toxicity

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Abstract: Invertebrate interactions like predator-prey relationships, competition for resources, mutualism and commensalism play an important role in the biogeochemical cycling of carbon and plant nutrients in soil. Besides direct impact on individual organisms, insecticides may also have detrimental effect on these crucial species interactions and, hence, the entire food-web in the soil. Despite of this, studies on how contaminants affects the structure and functioning of food webs in terrestrial ecosystems are very scarce. A soil multi-species (SMS) test system, consisting of a limited number of soil invertebrate species, with well-defined ecological roles, provides the basic characteristics of the soil ecosystem and enable us to study specific ecological topics, e.g. functional redundancy and species diversity. The aim of this study was to test the performance and validity of such a SMS test system by exposing invertebrate species and the microbial community to the insecticide α-cypermetin. In addition, we evaluated how two alternative worm species would affect the sensitivity of the SMS system. In this way two different faunal communities were constructed, each with three trophic levels and including five species of springtails and the predator mite *Hypoaspis aculeifer*. They only differed concerning the added oligochaete species, which was either juveniles of the earthworm *Eisenia fetida* or adults of the enchytraeid *Enchytraeus crypticus*. After 8 weeks, the EC50 was 0.76 mg kg^{-1} for enchytraeids and ranged between 2.7 and 18.9 mg kg^{-1} for collembolans, showing higher sensitivity than previously observed with single species. *E. fetida* had a positive effect on the majority of the species, apparently reducing the negative insecticide effect. *E. fetida* influenced the species sensitivity and decreased the degradation of the insecticide due to the organic matter incorporated as earthworm food. Changes observed in the community structure and function illustrates the strength of a SMS test system as an ecotoxicological tool compared to single species tests. **Key words**: Soil multi-species experiment, Chemical fate, Insecticide, Species interactions, Bioturbation.

Community and ecosystem ecotoxicology (I)

353

Characterizing the role of pesticides impacting surface water ecosystems in multiple stressed environments

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Groundwater and surface water resources are increasingly under pressure from global exploitation and anthropogenic impacts, such as contamination by chemicals. In response, the European Water Framework Directive requires member states to evaluate all types of contamination sources within a watershed in order to assess their direct impact on water quality. The clear linkage between these two systems requires assessments of "good" chemical and ecological status to include an evaluation of the contribution of toxicants entering surface water via the groundwater pathway. Pesticides are among the most harmful class of compounds impacting surface waters, particularly since they have been so widely used to control the occurrence of pests and weeds in urban and agricultural landscapes. In order to properly identify and subsequently mitigate the impacts to surface water ecosystems, a better understanding of the sources and types of pesticides expected in surface waters becomes essential. Here we (i) assess current trends for pesticide concentrations in surface water and compare them to the historical use of pesticides in Denmark (using sales data), (ii) investigate the importance of groundwater as a pathway for pesticide impacts to ecosystems, and (iii) quantify the impact of sediment-bound pesticides affecting stream macroinvertebrates. Notably, sediment-bound pesticides have long been disregarded as an important source of ecotoxicity, due predominantly to an assumed reduction in bioavailability. Results show that there is little correlation between pesticide findings in streams and estimated current use of pesticides. Chemical toxicity analyses identified sediment-bound insecticides such as chlorpyrifos and hexachlorobenzene as the primary source for ecotoxicity in the studied streams. The presence of the groundwater pathway, however, indicates that stream sampling during base-flow conditions can provide valuable information about the long-term fate of pesticides in groundwater. Ideally, integrative approaches should be developed with monitoring strategies simultaneously involving chemical analyses, ecotoxicological tools and the study of population/community responses in order to obtain a more holistic picture.

354

Comparing Single species Toxicity Tests to Community-Level Effects of Excess Total Dissolved Solids Doses Using Model Streams

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From 2011-2013, model stream chronic dosing studies (42 d) were conducted with three different total dissolved solids (TDS) recipes. The recipes differed in their relative dominance of major ions. Each was dosed to continuous flow-through model streams at the USEPA's Experimental Stream Facility (ESF), in Milford, OH. The community-level model stream dosing designs were also paired with single-species exposures conducted at both the bench scale using the whole effluent testing (WET) format and a new ex-situ exposure format. Single species exposure sensitivities were compared to whole community adult insect emergence response. We consider the latter a metric of whole community growth/development response. The mayfly, *C. triangulifer*, tested in the WET or in the ex-situ format was more sensitive to excess TDS regardless of the TDS composition than the other standard test organisms used; *C. dubia, H. azteca, and P. promelas*. Sensitivity of *C. triangulifer* tended to decrease in the ex-situ format, while the mayfly's sensitivity in the ex-situ exposure condition reflected that derived from mayfly emergence at the community scale. The combined exposure approach is designed to lend community-level context and more dynamic, field-like exposure conditions to help validate and evaluate endpoints obtained from more traditional single-species methods conducted in isolation at the bench-scale. Caddisfly emergence appeared generally as sensitive to the TDS exposures as mayflies, although less sensitive when the excess TDS is dominated by salts of Cl^-. Insects of the dipteran family Chironomidae dominated the total emergence response. Chironomids were less sensitive to excess TDS compared to the Ephemeroptera and Tricoptera taxa when the TDS was dominated by Cl^- salts and $NaHCO_3$ but appeared more sensitive when the excess TDS was a mixture of SO_4^{2-} salts and HCO_3^-. These results help to validate inferred sensitivities of stream insects to excess TDS from single-species exposures, qualifying existing State-level TDS targets for water quality protection, while also supporting the use of *C. triangulifer* WET tests for assessing exposures to excess TDS. The views expressed in this presentation are those of the authors and do not represent those of U.S.EPA.

355

Site recovery in the Zn-Pb mine of Olkusz, Poland, as shown by changes in the soil properties, nematode communities and plant tolerance

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This study focuses on environmental recovery on mining waste heaps of varying age, situated near Boleslaw and Olkusz in Southern Poland. The soil pollution with heavy metals (HM) in this area is one of the worst recorded in Europe with Zn concentration frequently exceeding 10'000 mg kg^{-1}. Bioindicators of food web complexity (e.g. nematode communities) and colonization by pioneer plant species are important signs of recovery at HM-contaminated sites. In Olkusz, metallicolous populations of the pseudometallophyte *Biscutella laevigata* L. (Brassicaceae) have evolved high HM-tolerance but do not hyperaccumulate HM. Here we combine nematode counting and identification, plant physiological and chemical analyses, and measurements of soil physical and chemical properties to pursue two principal objectives: 1) to document former Zn-Pb mining site recovery and reclaiming by natural ecosystem communities, 2) to analyse allocation–based Zn-tolerance in foliage and its variation as a function of site age. Preliminary results indicate slow site recovery, which appears to happen independently of site age. For instance, soil profiles show missing or weak incorporation of organic matter and the nematode communities at all study sites are still poorly developed. The nematode community structure indicates stressful environmental conditions with i) degraded food web structure, ii) high C:N ratio and iii) decomposition pathways dominated by fungi. Moreover, the average Zn concentration in above-ground and root organs of *B. laevigata* exceeded 1'000 mg kg^{-1}. This level of contamination may impede the development of herbivore communities. Further insights into the tolerance strategies of *B. laevigata* are expected from ongoing analyses on Zn microlocalisation in foliage. From the above results we conclude, that adaptations of local populations to a toxic environment, soil evolution ensuring successful vegetation colonization and metal-enriched dust immissions, rather than site age, likely determine the dynamics of environmental recovery at Olkusz.

356

Can soil communities and decomposition process in boreal forest recover from lead derived stress at a shooting range?

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An ecosystem-scale research was conducted in a shooting range area to study the fate and effects of lead on pine forest ecosystem and the recovery of the system 20 years after abandonment of the shooting range. Bioaccumulation, leaching and vertical distribution of Pb in soil, as well as decomposer communities and litter decomposition were compared between two contaminated sites (active [NC] and abandoned [OC] shooting ranges) and a control site, each site locatingin the same forest. The total Pb pellet burdens at the contaminated sites were similar, reaching up to 4 kg m^{-2}, and the shooting activity had lasted for approximately 20 years at both sites, but occurred 20 years earlier at OC. Total Pb concentrations at the shooting ranges were extremely high, and Pb also accumulated in the biota. The vertical distribution of Pb in the organic soil horizon differed between the shooting range sites, Pb concentrations at NC being higher in the upper fermentation (F) layer than in the lower humus (H) layer and at OC vice versa. Leaching of Pb was greatest in late autumn, being twice as high at OC than at NC. All faunal groups (total number of enchyraeid worms, microarthropods and nematodes) except protozoans were affected negatively by Pb, and the effects were most pronounced in the autumn. Also nematode and microarthropod community structures were affected. In the whole organic soil layer, the negative effects of Pb were less abundant at OC than at NC, especially among microarthropod taxa. Contradictory, in H layer the effects were stronger at OC, enchytraeid worms being completely absent. Pine needle litter decomposed at a lower rate at NC than at OC. The decreased total Pb concentrations in the top soil, decreased toxicity of the organic soil layer and the enhanced litter decomposition rate at the abandoned shooting range (OC) – when compared to the active shooting range (NC) – indicate an on-going recovery process at the abandoned shooting range. In boreal forest soil with low decomposition rate and intensity of soil mixing due to the scarcity of earthworms, a less contaminated top soil layer is gradually formed when shooting activity is ceased. This layer can provide habitats for decomposer biota and promote the recovery of soil functions. However, at the same time the dissolution of Pb from the pellets deeper in the soil increases the toxicity of humus and leaching of Pb posing risk to the ecosystem and groundwater quality.

357
Co-occurrence patterns as a response of interaction network of terrestrial microbial communities to atmospheric pollution and seasonal variations
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The ability of ecological systems to continue to deliver the ecosystem services on which human well-being ultimately depends is being increasingly compromised by anthropogenic activities. Traditional holistic works are restraint to food-web ecology while co-occurrence patterns provide a good tool for revealing networks with all interactions among organisms. Microbial communities provide a particularly favorable model to study the community structure, its link to ecosystem functionning and its response to environmental conditions through time. In this study, we evaluate the impact of chemical (particles or gaseous) pollutions on interactions among microbial genera and ecosystem functionning by co-occurrence networks modelling for microbial communities associated to bryophytes under three different polluted conditions (rural, urban and industrial) over an 8-month period. A first result of this study is the change in the community structures linked to the seasonal variations (autumn vs. spring) but not obviously among sites. The genus richness varies significantly among the sites (25 in average at rural site, 20 in average at polluted sites). Our result suggests the diversity is more affected by the pollution stresses whereas the structure of community is essentially sensitive to seasonal variation. The network models and indices calculation showed an evolution of the complexity of the relationship among microbial genera through time and seasons. We observed a modification of the interaction quality between nodes (e.g. negative to positive or positive to negative) and a modification of the genera centrality, according to the type of pollution. The addition of environmental conditions in the co-occurrence patterns indicates which the co-occurring assemblages of organisms prefer or avoid and the inclusion of chemical stress measurements may link groups of organisms with particular functions.
Co-occurrence analysis provides an essential view of the real-world ecosystem response to anthropogenic stresses. Key words : ecological interactions, modelling, microorganisms

358
Seasonal variation in effects of different land-uses on stream ecosystem structure and function
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Stream ecosystems are impacted by a variety of anthropogenic stressors originating e.g. from agricultural activity, urban areas and wastewater treatment plants. As a result, the structure of aquatic communities as well as associated ecosystem functions (e.g. leaf litter breakdown) may suffer from this contamination. Therefore, the present study assessed alterations in structural (invertebrate community composition) and functional (microbial and invertebrate mediated leaf litter breakdown as well as *in situ* bioassays with *Gammarus fossarum*) endpoints along a low order stream in Southern Germany during winter 2010/11 and summer 2011. This stream is gradually impacted by viniculture, urban area and motorway runoff as well as wastewater release. Microorganism mediated leaf litter breakdown was – possibly due to elevated nutrient concentrations – significantly increased by up to 270% at sites downstream of the city, motorway and wastewater treatment plant during winter but unaffected in summer. At the same sites, macroinvertebrate mediated leaf mass loss was significantly reduced during winter (up to 100%) and summer (up to 44%) compared to the reference site. This effect may be explained by changes in the macroinvertebrate communities, especially by the reduced abundance of a key leaf shredding macroinvertebrate, i.e. *Gammarus*. This reduction may be triggered either indirectly (altered food quality) as indicated by a significantly reduced fungal biomass (up to 58 and 70% during winter and summer, respectively) associated with leaf material, which may have influenced the physiological fitness of shredders. On the other hand, direct ecotoxicological effects of water contamination are also conceivable as gammarids exposed *in situ* exhibited a significantly (up to 55%) reduced feeding rate downstream of the urban area, the motorway (both only during winter) and the wastewater treatment plant (during both seasons). Such reactions in feeding are indicative for a reduced reproductive output in the long run. Finally, this study urges that besides water pollution arising from agricultural activity and wastewater release, the influence of (seasonal) pollution from urban areas should be adequately considered in the future to achieve the "good ecological status" required by the EU Water Framework Directive.

Biodegradation and Environmental Fate of Chemicals - Regulatory Acceptance of Non-Standard Tests

359
Revisiting the evaluation process of biodegradation of chemical substances: the ProbaBio probability concept.
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For the registration of a chemical substance, the European REACH regulation requires, *inter alia*, the evaluation of its biodegradability. A large set of test methods is available since 1992. However, biodegradation results for molecules published in literature, show intra and inter laboratory incoherencies linked to the microbial community variability. Indeed, if biodegradation is achievable from the growth of specific microbial degraders with a substance as the sole source of carbon, the result of the test depends largely on the initial density of degraders. If these species are relatively rare in an environment the likelihood of inoculating a test with sufficient specific cells becomes a matter of probability. It seems then possible to reach a probability of the environmental persistence of an organic compound. In the alternative ProbaBio approach developed in this study, the persistence is not only regarded as a simple intrinsic substance property, but also as the potential presence of specific degraders in various environmental samples (activated sludge, river water and sediment, soil and sea water) to degrade a chemical under realistic exposure (incubation temperature, biomass density at time zero and temporal shifts due to potential seasonal influences). A miniaturized O_2 sensing platform has been developed for a high throughput screening to qualify the biodegradation event of readily to persistent molecules (5700 tests). Results show the probability, represented as environmental biodegradation fingerprints, of five molecules to be degraded according to the environments and its conditions. The ten environments tested have not the same potential of biodegradation. Incubation temperature impacted directly the fate of the biodegradation tests in two ways, (a) the time window required in ready biodegradability tests (RBT) cannot be reached at lower temperature (12°C) and (b) the pass level was reached only at 25°C excluding from the RBT standards the same molecules at 12°C. Moreover, there was a seasonal effect on the biodegradation tests results, thus, some molecules were biodegradable in summer and not in winter. The biomass density at time zero plays the same effect as the temperature. To determine the link between all this parameters, all the tests have been statistically treated. This approach is a new proposal to improve our knowledge about the difficult task of biodegradation assessment.

360
Enhanced Regulatory Assessments within REACH
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Chemical compounds are ubiquitous within the aquatic environment, causing concern over human health and the environment. The principal elimination process for their removal is microbial degradation, the prediction of which plays an important role in risk assessment. Historically, ready biodegradability tests (RBTs) have formed the core protocol for developing regulatory guidelines. Recently, however, regulatory concerns have developed at a faster rate than standard tests with an increased emphasis placed on approaches to identify Persistent, Bioaccumulative and Toxic (PBT) chemicals. RBTs are notoriously variable, producing a large number of false negatives. The Registration, Evaluation, Authorisation and restriction of CHemicals (REACH) was introduced in 2007 to streamline chemical legislation within the European Union (EU). REACH stipulated the same data requirements for new and existing chemicals, necessitating the development of reliable and reproducible screening tests capable of accurately prioritising chemicals for further testing. A series of enhancements and modifications to existing biodegradation screening tests (BSTs) were proposed as a result of a workshop on biodegradation and persistence hosted by the European Centre for Ecotoxicology and Toxicology of Chemicals, which were adopted in principle within REACH to enable a more effective prioritisation on persistence. The proposed enhancements of increasing test duration, test volume and cell concentration were investigated. The present study assessed methods for the concentration of bacterial cells in aqueous environmental samples. Analysis of scientific and practical criteria were used to inform the selection of the most suitable concentration method for investigations into the effect of volume and cell concentration in activated sludge and marine BSTs and the subsequent development and validation of enhanced BSTs using activated sludge (AS) and marine inocula. Enhancements in AS BSTs were found to significantly reduce variation between replicates ($P < 0.01$), with coefficients of variation as low as 0.34% when using larger test volumes and higher cell concentrations. Enhanced marine BSTs failed to significantly reduce variation between replicates and suggested the requirement for further work, perhaps focusing on investigating pathways and mechanisms of adaptation. The application of molecular techniques and use of predictive models to facilitate future chemical assessments is also discussed.

361
Repeat of previously conducted ready biodegradability studies following current regulatory guidance, and its effect on persistence assessment
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The performance of ready biodegradability studies on poorly water-soluble chemical substances has been recognized as problematic for several years. ISO (International Standards Organization) came out with guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability (ISO, 1995). However, studies continue(d) to be conducted not utilizing this guidance. Ready biodegradability studies conducted in the 1990s (and in some cases, after as well) often also used test substance (TS) concentrations several times the water solubility limit, ignoring the fact that the TS was not really bioavailable to the bacteria at these concentrations, and reported no biodegradation. Often, these studies were done to the OECD 301C guidelines which arguably, stipulate the most stringent conditions to test ready biodegradability. Using results from such studies as such, to evaluate persistence from such studies, for PBT (persistence, bioaccumulation and toxicity) assessment under REACH, meant that there was a very high probability that the substance would get a 'P' label, whether it deserved it or not. Since the ISO guidance was published, there has been significant research in this area by many workers (Handley, et al: 2002, van Ginkel, et al: 2008, etc.). ECHA (European Chemical Agency) too has included this consideration for biodegradability testing of poorly water-soluble substances in their endpoint specific guidance (ECHA, 2012). Using favorable conditions to make the TS more bioavailable has shown to improve biodegradability for such 'difficult-to-test' substances and is being accepted by regulatory authorities, as more representative of what occurs in natural environmental systems. This presentation will include a case study of a substance with very low water solubility, that was deemed to be P or vP based on a 1996 Japanese study that showed very little to no biodegradability. TS concentrations used in this study were almost two orders of magnitude higher that the known water solubility. When the study was repeated in 2011 using 'modified' conditions to make the TS more bioavailable, the TS showed up to 46% biodegradation.

362
Considerations when using OECD 301 tests to assess 'degradation' of hydrocarbon products
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Degradation of organic chemicals in the environment influences exposure and, hence, it is recognised as a key parameter for estimating the risk of long-term adverse effects on biota. The OECD Guideline 301 A-F tests are used to assess the ready biodegradability of organic chemicals. The results of these tests are used extensively both within a regulatory context (i.e. chemical hazard assessment) and by industry to support marketing claims on product biodegradability (i.e. 'green credentials'). However, a fact that is often overlooked is that these stringent tests were developed as relatively simple and inexpensive methods to screen organic chemicals for ultimate biodegradation over a period of 28 days. In the OECD 301 A-F series of aerobic tests, the biodegradation of a high concentration of the test substance (in the range of 2 to 100 mg/L) is measured by non-specific parameters such as loss of Dissolved Organic Carbon (DOC), or the extent of respiration increase (O_2 uptake measured as Biochemical Oxygen Demand (BOD) and CO_2 evolution) over time. These OECD 301 tests have several limitations, particularly when used to assess the biodegradability of complex and/or poorly water soluble substances. In this presentation we provide an overview of some of the recent experiences encountered with the conduct and interpretation of OECD 301B and F tests undertaken on complex hydrocarbon products. We also discuss some additional steps which can be taken to improve the conduct and reporting of such studies when used to assess the biodegradation of hydrocarbon products such as fuels and lubricating oils.

363
Improved test system to determine chemical degradation in laboratory water/sediment systems – experimental results
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The OECD 308 guideline on "Aerobic and Anaerobic Transformation in Aquatic Sediment Systems" has been used since 2002 extensively in different legislative frameworks. However, several shortcomings have been identified and discussed over the years on the guideline: the combination of degradation and sorption, which makes it impossible to locate where and how degradation occurs; the unrealistic water-sediment ratio of 3:1 which often result in high levels of non extractable residues (NER) and huge anaerobic areas in the sediment. Thus, a derivation of a compartment specific degradation half-live is hardly possible from OECD 308. In the last years the OECD 309 guideline for biodegradation in aerobic natural waters, became more important. It is run at low sediment concentrations under fully aerobic conditions as a laboratory shake flask test. With concentrations between 0.01 and 1 g suspended solids (SS)/L it might potentially exhibit several advantages over OECD 308 for determining biodegradation. Against this background, the aim of the Cefic-funded project LRI-ECO18 is to understand the value and information content of the existing OECD 308 and 309 protocols and to develop an improved testing strategy to obtain robust degradation in water/sediment systems. In combination with a data analysis approach, an experimental approach is used to enable disentangling sorption from (bio)degradation and clearly distinguish between aerobic and anaerobic conditions. A suite of four different water/sediment systems is used to investigate the behavior of four chemicals with varying sorption properties and biodegradability: (1) OECD 308 standard protocol (water:sed ratio = 3:1, not stirred); (2) OECD 308 modified protocol (water:sed ratio = 10:1, stirred water phase); (3) OECD 309 modified protocol (water:sed ratio = 100:1, stirred system); (4) OECD 309 standard protocol (water:sed ratio = 1000:1, stirred system). The presentation will focus on experimental methods and the current status of data determination including first results and conclusions about the influence of the experimental setup on substance degradation. The variation of the test systems might help to improve the experimental setup and to overcome some of the criticism on the current OECD 308. In combination with advanced parameter estimation techniques the Cefic project will provide a tool to better understand the processes at the water-sediment interface.

364
Estimating transformation rate constants from OECD 308 data – Opportunities and limitations of existing data
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The OECD guideline 308 for the testing of transformation in aquatic sediments is an integral part of environmental risk assessment of chemicals. The results of these studies are generally used to derive half-lives in water phase ($DT_{50,w}$) or in the whole experimental system. However, both of these values depend heavily on the loosely standardised system geometry. Moreover, $DT_{50,w}$ lumps diffusion and transformation rates together. Overall, none of these indicators seems to be well suited for assessing actual transformation rates or – in general – the persistence of chemicals. In the Cefic-funded LRI-ECO18 project we used Bayesian parameter estimation with four different model versions that represent the experimental system with different hypotheses about degradation and diffusion in the sediment. The models were calibrated to existing data for 25 pesticides and pharmaceuticals. Available information was used to support model calibration in form of parameter prior distributions. All 3 model versions explicitly describing diffusion in the sediment could fit the data well, which indicated that experimental data do not provide evidence about the depth-dependence of degradation in the sediment. As a consequence, calibrated sediment degradation rate constants differed strongly by model version, whereas degradation rate constants in water could be estimated more robustly. Degradation half-lives in water, $DegT_{50,w}$, as derived from the calibrated degradation rate constants were significantly longer than the disappearance half-lives $DT_{50,w}$ estimated from the raw data.

Identification and prioritisation of hazardous emerging pollutants (I)

365

Integrated approach for the identification, prioritisation and abatement of emerging pollutants – a SOLUTIONS - oriented approach

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Despite the extensive efforts to attain a good quality status for all European river basins, in the majority of cases this goal has not been achieved. There are strong indications that toxic chemicals are significantly responsible for this failure although the quality status of rivers could not be related to the chemical status defined by 41 priority and other pollutants. This may be due to the enormous complexity of chemical pollution with more than 100.000 chemicals in daily use and mixtures of tens of thousands of chemicals in typical environmental samples. Thus, this paper presents a novel conceptual framework to provide solutions to the contamination problems associated with legacy, present and future pollutants in European water resources, developed by the European Collaborative Project SOLUTIONS. This framework integrates models and tools for identification, prioritisation, assessment and abatement of hazardous emerging pollutants with respect to ecosystems and human health. The framework is designed as the basis of a user-friendly decision support tool that helps to identify trains of models and tools for the identification of River Basin Specific Pollutants and of appropriate abatement options. This approach is demonstrated in large trans-European case studies in the Danube, Rhine and several Iberian river basins. First results of pollutant identification and prioritisation in the largest SOLUTIONS case study, based on the Joint Danube Survey 3 organised by the ICPDR, will be presented and discussed along with concepts developed and open research questions raised. The new data and information on emerging pollutants will be compiled in an extensive knowledge base linked to the European integrated data platform on chemical monitoring data (IPCheM).

366

An innovative and comprehensive study to identify relevant emerging contaminants in French surface waters

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As part of the implementation of the National Action Plan on Micropollutants in the Aquatic Environment, the French Ministry of Ecology a large national screening study took place in 2012 in France. INERIS was charged with the design and technical implementation of this project for surface water. For the selection and prioritisation of the watch list compounds the National Expert Group for prioritisation of substances (CEP) decided to adopt the criteria of the NORMAN methodology for prioritisation of emerging substances.That methodology uses a decision tree that first classifies chemicals into six categories, on the basis of the existing knowledge gaps and the actions to be taken to fill them. The priority within each category is then ranked on the basis of specific indicators, which allows a score to be calculated. For surface water three campaigns were performed on water matrix and one in sediments at about 159 sampling points. Grab sampling was applied on all sites. In addition, passive samplers (POCIS) were deployed in 20 rivers in order to allow the implementation of bioassays. Sampling was carried out according to AQUAREF's (National Reference Laboratory for Aquatic Environment) technical specifications and a unique courier was selected for transport of samples to the laboratories. The analytical work for a given group of compounds was performed by one single laboratory in order to ensure data comparability. Four academic laboratories were selected for chemical analyses. 170 substances were finally selected for chemical analysis in water and sediment.The benefits of this innovative approach for identification of relevant emerging contaminants are i) a unique prioritisation scheme (NORMAN Network) applied at French national level for substance and site selection; ii) Information on occurrence, source and variability for 170 substances (50 000 robust analytical data items) ; iii) an efficient method for data collection, validation and exploitation thanks to a unique data collection template and an exhaustive set of metadata; iv) the application of effect-based tools to classify 20 sites of various quality; to characterise site contamination a panel of *in vitro* and *in vivo* bioassays were deployed as well as biomarkers to assess impacts of pollutants in wild population of fish.The results contributed to the selection of substances to be integrated in the national river basins monitoring programmes.

367

How much risk can be covered by monitoring prioritized substances in surface waters? A complete pesticide screening example

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Synthetic pesticides have been used in agriculture and in urban areas for many decades. The risk that pesticides can pose to surface waters was recognized a long time ago and subsequently critical substances were banned and/or listed as priority pollutants, for example in the European Water Framework Directive (WFD). Because new pesticides are entering the market every year and pesticide application varies regionally, river basin specific pollutants should, according to the WFD, be additionally selected. However, when pesticides are prioritized and only the selected substances are monitored, there is always the chance to miss potentially important substances. The goal of our study was to perform a complete analytical pesticide screening in five agriculturally influenced rivers during the main pesticide application period, assess the associated risk for the aquatic environment, and compare it with selected subsets of pesticides which are regulated (WFD) or selected for monitoring in Switzerland. The analytics were done using solid phase extraction (SPE) liquid chromatography coupled to high resolution tandem mass spectrometry (LC-HRMS/MS, QExactive). By using a combined target and suspect screening method, 86% of all registered synthetic organic pesticides (310) could be detected in low concentrations (0.5-10 ng/L). In addition, for 10 highly toxic pyrethroids, a GC-MS/MS method was set up using polydimethyl-siloxan (PDMS) passive samplers. In the complete analytical screening, 30-40 substances were detected per sample and the sum of pesticide concentrations was on average 2 to 4 μg/L. The sum of individual risk quotients following the concept of concentration addition for plants and invertebrates exceeded the critical value by a factor of 4-6 and 2-3, respectively. It was found that only 50% of the detected substances would be measured when a subset of only 30-40 pesticides is investigated, but that the sum of pesticide concentrations was not strongly influenced. Applying the mixture toxicity approach (sum of individual risk quotients), the scenarios would on average (whole investigation period in the five rivers) miss 30-40% of the total risk. This number, however, varies between the different rivers. Thus, if priority substances are selected carefully, they can in most cases (in medium sized rivers) sufficiently cover the risk of surface waters. However, to really assess the complete exposure of pesticides, a full chemical screening is essential.

368

Are carboxylic acid metabolites from alkylated PAHs a new class of potential contaminants in marine waters?

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Concentrations of alkyl substituted PAHs in crude oils are much higher than concentrations of nonsubstituted PAHs, and certain C1-C4 alkyl substituted PAHs are highly toxic. In spite of this knowledge, only little attention regarding ecotoxicological fate and effects of these compounds have been given in research. We have previously shown that the benthic invertebrate Nereis diversicolor metabolize 1-methylpyrene in sediment exposure setups more than five times faster than its non-alkylated relative, pyrene. In addition, we showed that this rapid metabolism primarily results in production of the water-soluble aromatic acid, 1-pyrenecarboxylic acid (90% of the total metabolites). Here we show that also methylated phenanthrenes and chrysenes exposed to N. diversicolor generate aromatic acids as main metabolites. In addition, we show that metabolism is faster for alkylated PAHs than for their relative non-alkylated PAH. Our results suggest that metabolism of crude oil spilled to benthic environments in general results in a build-up of water-soluble aromatic acids.

369

Occurrence, risk assessment and prioritization of emerging pollutants in Mediterranean (Iberian) rivers

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Introduction Worldwide contamination of aquatic systems with numerous chemicals is one of the key environmental problems. One approach for identifying dangerous compounds is screening of the environment for a large set of chemicals together with an assessment of the potential toxicity.Within the project SCARCE-Consolider project an extensive sampling at 77 sites from four Iberian river basins was undertaken. This work presents a synthesis and critical evaluation of levels for 244 organic emerging contaminants in water and provides a basis for assessing the risk towards ecosystem for each river basin. This also allows providing a prioritization list of these contaminants in Iberian rivers that can be relevant from the regulatory point of view. **Results** The most frequently detected chemicals were of industrial origin, followed by personal care products parabens, degradation products of industrial detergents, some pharmaceuticals such as azitromycin, and pesticides chlorphyriphos and diazinon. In the highest average concentrations were found: nonylphenol monocarboxylate, tolyltriazole, tris(chloroisopropyl) phosphate found at average concentrations above 100 ng/L. Llobregat river was the most contaminated, showing clear and significant increase in concentrations downstream the river being the sites near the mouth of the river the ones with the highest contaminants load. In the Ebro river basin several hot spots can be identified especially where treated wastewaters are discharged into the river or its smaller tributaries. Of studied river basins, Jucar showed to be the least contaminated. At majority of sampling sites pesticides contribute to the major extent to the overall risk, with the exception of lower Llobregat where significant contribution from perfluorinated compound and smaller from pharmaceuticals is observed and several points at Guadalquivir with significant contribution from EDCs of industrial origin. In terms of individual compounds posing the highest risk highlighted were prochloraz, diuron and triclosan. **Acknowldegment** The authors wish to acknowledge the financial support provided by the Spanish Ministry of Science and Innovation through the projects SCARCE (Consolider-Ingenio 2010 CSD2009-00065) and CEMAGUA (CGL2007-64551/HID) HID), and the Generalitat de Catalunya (Grup Consolidat de Recerca: Unitat de Qualitat de l'Aigua i Sòls, 2009SGR965). Maja Kuzmanovic acknowledges AGAUR fellowship

370

Forensic ecotoxicology in a multi-stressed Mediterranean river: unravelling major toxic effects of phytotoxins in a superfund site in Ebro River (NE, Spain)

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Assessing and identifying toxic compounds in multi-stressed rivers in the field require the combination of toxicity assays and chemical analytical/fractionation methods. Among the existing procedures, TIE (Toxicity Identification Evaluation) approaches are among the most accepted ones but many times are unsuccessful in detecting causal-toxic compounds within complex samples. Lack of success often occurs when toxic effects are associated to a minor compartment fraction of the sample (i.e. suspended material within water samples), effects are sublethal, toxicity is due to low amounts of highly toxic compounds and/or when toxic compounds are very polar. Here we present data on a long term study performed to determine the toxic components present in the suspended material fraction of the water column. The study was conducted in the lower part of Ebro River (NE, Spain) at the Flix superfund reservoir site during an unusual prolonged period of high water flow with an increase two to five fold (January to June 2013). Flix reservoir is heavily contaminated with organochlorine and mercury wastes deposited in the sediment from a chlor-alkali industry operating for more than 50 years. The study aimed to assess how water flow increases would affect the re-suspension of toxic sediment wastes into the water column. Chemical compounds of suspended material were extracted with a battery of non polar and polar solvent and analysed by GC-MS/MS. Mercury content was also determined for all sites. Post exposure feeding *Daphnia magna* rates were used to asses toxic effects of whole water samples and of the filtered water and re-suspended solid fractions. Organochlorine and mercury residues in the water samples increased from upstream to downstream locations. Conversely, toxic effects were greater at the upstream site than at and downstream of the superfund Flix reservoir. A further analysis of the suspended solid fraction identified a toxic component eluted within the 80:20 methanol:water fraction. Characterization of that toxic component fraction by LC-MS/MS identified three different candidate phytotoxins coming from the bottom sediment upstream and re-mobilized when opening the reservoir dams. This study provides evidence that, in real field situation under the action of multiple stressors, measured contaminant residues not always agree with toxic effects.

Recent advances and critical future research directions for poly- and perfuorinated alkyl substances (PFASs) (II)

371

Substitution of prioritized poly- and perfluorinated chemicals to eliminate diffuse sources (SUPFES)

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Fluorinated substances have important technical applications in consumer products, e.g. in textiles where they are used for oil and water repelling properties. The aim of the SUPFES project is to help industry find alternatives that can replace poly- and perfluorinated chemicals (PFASs) which are harmful to the environment. Within the project a number of scientific and industrial partners collaborate to assess the risks with different chemicals and make sure that the new alternatives provide desired functionality. PFASs have important technical applications in consumer products, e.g. in textiles where they are used for oil and water repelling properties. However, some PFASs are long-lived and accumulate in the environment. In several independent studies they have been found to cause adverse effects on animals' development and negative changes in humans' immune systems. The substances have been found in the blood of humans, mammals, sediment and water in very remote areas such as the Arctic. Some PFASs are already banned under UN regulations (e.g. PFOS) and regulated in the European chemical legislation REACH (e.g. PFOA). A unique consortium has been established in connection with the SUPFES research project, consisting of: Swerea IVF, Chalmers University of Technology, VU University Amsterdam, Stockholm University, Käppala wastewater treatment plant and outdoor retailer Haglöfs. The SUPFES project will test the hypotheses that emissions of these chemicals are mostly generated as diffuse emissions, i.e. as small and large emissions throughout the whole life of the product, and that textiles are an important source of these emissions. Alternative chemicals will be tested on functionality, leaching behaviour, toxicity and a life-cycle assessment will be performed.

372

Perfluorinated alkylated substances in freshwater fish on Svalbard

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Perfluorinated Alkylated subsances (PFASs) were analysed and quantified in Arctic char (*Salvelinus alpinus*), from the lake Linnévatnet in Svalbard in order to investigate levels and distribution of this substances in a typical Arctic freshwater system. In many high Arctic region, Arctic char is the only resident fish species in freshwaters, and therefore favourable as biomonitoring species also for this project. During the fieldwork in March 2013 (N=6, liver and fillet samples) and September 2010 (N=13, fillet samples) Arctic char were caught and kept frozen until analyses of PFAS. The analyses were conducted at the Norwegian Institute of Air Research (NILU) in Tromsø. Principal component analysis (PCA) was used to investigate the relationship between the samples and the PFASs. The results confirmed that perfluorobutanoic acid (PFBA) and perfluorohexanoic acid (PFHxA) were the most abundant contaminants detected in all samples. In 2010, PFBA in fillet samples showed the highest concentrations (in ng/g w.w). The fluortelomer 6:2FTS showed highest concentration in the liver samples from 2013. The short-chained PFASs compounds (4-6 carbons) contributed to 78 % of ΣPFAS in the 2010 measurements, while the long-chained PFASs (7-13 carbons) together accounted for 56.8% of the ΣPFAS in 2013. In general, the concentrations were relatively low. However, there was a decrease in levels of PFASs in fillet samples from 2010 to 2013, while the highest levels were mainly detected in liver samples from 2013. This is consistent with previous studies indicating that PFAS bind to protein in the blood and bioaccumulate in the liver. Because of the location of the lake Linnévatnet and the low concentrations of PFASs, long-rang atmospheric transport is the main pathway for these contaminants instead of local sources. Based on previous studies, perfluorooctanoic sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were expected to have higher concentrations and to be the dominating contaminants, but this was not the case in the present study. Detecting levels of PFASs in Svalbard, which are being used in production of perfluorinated products in Asia, give reasons for further research and to investigate temporal trends and distribution of these contaminants in the European Arctic.

373

Accumulation and elimination of perfluoroalkyl substances (PFASs) by the insect (Chironomus riparius) larvae (Diptera, Chironomidae) exposed to sediment

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Long chain perfluoroalkyl acids or sulfonates and a few precursors have been found in different media of the Rhone river downstream of Lyon (France). Although most of these compounds are considered as bioaccumulative, the exposure pathways of aquatic biota and the accumulation kinetics remain poorly understood. The objectives of this study are (i) to assess the accumulation and depuration kinetics of C_9 to C_{14} carboxylic acids (nonanoic PFNA to tetradecanoic, PFTeDA), C_8 to C_{10} sulfonates (perfluoro-octane, PFOS to perfluoro-decane PFDS) and 3 precursors, in a benthic invertebrate species, the midge *Chironomus riparius*; (ii) to test the concentration dependency of PFASs accumulation. Chironomids are an important food source for other species; their life cycle involves a larval stage in contact with sediment and use to feed on detrital carbon food source. Natural sediments from a deposition site along the Rhône river (France) downstream of an industrial site releasing amounts of PFASs were collected with a Van-Veen grab, sieved at 2 mm, pooled in polypropylene jars and stored at 4°C. Chironomids were exposed at their optimum temperature (21°C). Two series of experiments were conducted, dealing with uptake and depuration respectively. PFASs were analysed in water, pore water, sediment and organisms by LC-MS/MS. The accumulation of all tested perfluoroalkyl substances (PFASs) is measurable already at the first intermediate time (two days). Transfers from sediment to surface or pore water as well as chironomid larvae can be demonstrated. Biota to sediment accumulation factors appear lower than those published for *Lumbriculus variegatus* ([1,2]). These results allow to draw a conceptual model of PFASs distribution in abiotic compartments, and to determine the pathways and dynamics of accumulation by chironomids. Tentative uptake and depuration rates were calculated on the basis of the uptake experiment and a partition model ([1,3]). Experimental depuration rates will be presented, and the perspectives to develop a complete kinetic model will be discussed. ([1]) Higgins, C. P., McLeod, P. B. et al. (2007). Environmental Science and Technology 41(13): 4600-4606 ([2]) Lasier, P. J., Washington, J. W. et al. (2011). Environmental Toxicology and Chemistry 30(10): 2194-2201 ([3]) Landrum, P. F. (1989). Environmental Science & Technology 23(5): 588-595

374
Life-traits analysis for evaluation of long-term effects of perfluorinated compounds (PFASs) on Chironomus riparius (Diptera, Chironomidae) under a multi-generational approach
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A multi-generational test with *Chironomus riparius* was performed in order to assess long-term effects on life-traits of selected perfluorinated compounds, i.e. PFOS, PFOA and PFBS. These persistent contaminants are widespread in aquatic ecosystems and may cause long-term toxicity. Among aquatic invertebrates, *Chironomus* was shown to be sensitive to PFOS at low concentrations (NOEC = 21,7 μg/l, 20-d growth of *C. tentans*, MacDonald et al., 2004), possibly due to interaction with hemoglobin. Based on this result, in this study *C. riparius* larvae were exposed for 10 generations to 10 µg/l solutions of PFOS, PFOA and PFBS, i.e. at the maximum values found in Italian freshwaters. For each generation, the following life-traits were estimated: survival (emergence ratio), growth (weight of IV instar larvae, weight of male and female adults, length of male and female pupal exuviae), development (development rate, mean emergence time), and reproduction (number of egg ropes per female, number of eggs per egg rope, number of hatching eggs per rope). Comparison between treatments and control were performed for each generation. No effects on survival were found. Treatments showed reduced growth, with significant differences for PFOS and PFOA. Development was affected by PFOS and PFOA, with anticipated or delayed emergence. Reproduction traits showed no significant differences between control and treatments. PFOS showed effects mainly in the early generations, possibly inducing adaptative response of the population. PFOA showed effects in all generations, possibly inducing physiological response. PFBS affected only the last generations, i.e. the most stressed by the lab breeding conditions. These pre-exposed populations will be used for a final test on tolerance induction to 100 μg/l PFOS and 200 μg/l PFOA. Results show that PFASs can induce long-term toxic effects in aquatic communities at low concentrations, with different mode of action.

375
Are fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their precursors safer than the substances they replace?
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Long-chain perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkane sulfonic acids (PFSA) are persistent, bioaccumulative, and toxic contaminants that are globally present in the environment, wildlife and humans. Consequently, phase-out actions and use restrictions to reduce the environmental release of long-chain PFCAs, PFSAs and their precursors have been taken since 2000. In particular, long-chain perfluoroalkyl substances (PFASs) are being replaced with shorter-chain homologues or other fluorinated or non-fluorinated alternatives. A key question is: are these alternatives, particularly the structurally similar fluorinated alternatives, safe to humans and the environment? Over 20 fluorinated alternatives including a new type of perfluoroether carboxylic acids (PFECAs) have been recently identified. However, the scarcity of experimental data prevents comprehensive hazard assessments for these substances. In this study, we use state-of-the-art *in silico* tools to assess some properties of these newly identified fluorinated alternatives. [i] COSMOtherm and SPARC are used to estimate physicochemical properties including the octanol-water (K_{OW}), air-water (K_{AW}) partition coefficients, and acid dissociation constants (pK_A). The US EPA EPISuite software package is used to predict half-lives in air, water and soil. [ii] In combination with estimated chemical properties, a global-scale multimedia mass-balance model – the OECD Overall Persistence (P_{OV})and Long-Range Transport Potential (LRTP) Screening (OECD) Tool – is used to assess the likely environmental fate of these alternatives. Even though the fluorinated alternatives contain some structural differences, their physicochemical properties are not significantly different to their long-chain predecessors. Furthermore, most of the alternatives, e.g. the PFECAs, are estimated to be similarly persistent and transportable as the long-chain PFASs. The models therefore predict that the fluorinated alternatives will become globally distributed in the environment like their long-chain predecessors. The alternatives may also bioaccumulate in biota and humans, but it is not possible to accurately predict bioaccumulation of PFASs with existing *in silico* tools. Nevertheless, whilst such *in silico* methods are coupled with uncertainties, this preliminary assessment provides enough cause for concern to warrant experimental work to better determine the hazard properties of these fluorinated alternatives.

Biophysical Interactions at the Bio-nano Interface: Relevance for Aquatic Nanotoxicology (I)

376
A novel two-compartment barrier model for investigating nanoparticle transport in fish intestinal epithelial cells
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The rapid growth in development, production and use of engineered nanoparticles (ENPs) leads to a strong increase in both exposure of humans and the ecosystem to these new materials. However, the current knowledge of effects potentially caused by these new materials is still limited. We developed a new two-compartment intestinal barrier model using a fish cell-line and investigated its ability for studying nanoparticle transport through fish intestinal epithelium. Rainbow trout (*Oncorhynchus mykiss*) cells (RTgutGC) were successfully grown on transwell inserts with membranes of different pore sizes (0.4, 1 and 3 μm) leading to a two-compartment barrier model that allows to investigate the transport of ENPs through a model of fish intestinal epithelium. The cells form a polarized monolayer and express the tight junction protein ZO-1 as shown by immunohistochemistry. Measuring the transepithelial electrical resistance (TEER) reveals that the cells build up an epithelium with TEER values comparable to the *in vivo* situation. Incubation of the cells with fluorescent polystyrene nanoparticles (PS-NPs; average hydrodynamic diameter: 73 ± 18 nm) for 24 h lead to a very intense fluorescent signal in the apical medium indicating that most of the particles still remain in the apical compartment after the incubation period. However, there was also a detectable fluorescent signal in the cells, demonstrating accumulation of at least some of the PS-NPs in the cells. In contrast, the basolateral medium shows no significant fluorescence which indicates that a transport of PS-NPs through the cell layer does not occur. The situation is different when empty (cell free) inserts were used: Here, fluorescence in the basolateral compartment was detectable at least for the inserts with larger (1 and 3 μm) pore sizes. These results show that a transport of the PS-NPs by simple diffusion to the basolateral compartment is possible and thus validates the system as suitable for investigating nanoparticle transport. In addition, these results further demonstrate that the RTgutGC cells function as a barrier preventing the uptake of ENPs to the basolateral chamber that could be extrapolated to the blood stream *in vivo*. *Acknowledgement* – This research has been supported by EU FP7 grant NanoValid no 263147.

377
Citrate gold nanoparticle exposure in the marine clam Ruditapes philippinarum: Uptake, elimination and effect
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of Algarve; J. Blasco, Inst Ciencias Marinas de Andalucia / ECOLOGY AND COASTAL MANAGEMENT
Engineered gold nanoparticles (AuNPs) are introduced into a growing number of commercial products. The resulting increase in likelihood of release into various environmental compartments needs to be met by an increased understanding of their effects on the species within. Up to date limited information is available on the ecotoxicological risk for non-target organisms in marine environments, with the majority of research focusing on magnified expectable environmental concentrations. In our laboratory-based study the bivalve *Ruditapes philippinarum* was chosen as a model to evaluate uptake, elimination and sub-cellular effects of citrate-stabilized AuNPs (21.5 ± 2.9 nm) at an environmental relevant concentration (0.75 μg L^{-1}) over 14 days. Tissue (digestive gland and gills) was sampled on days 0,1, 7 and 14 to record changes in the expression of chosen endpoints over time. Gold concentration in tissue and faeces, enzymatic biomarkers of i.) oxidative stress (CAT, GST, SOD, GPx, GPx-Se), ii.) damage (lipid peroxidation, DNA strand breaks) and iii.) exposure (Metallothionein, AChE), as well as gene expression by means of quantitative reverse transcription PCR (qRT-PCR) for selected sets of genes were measured. We observed an uptake of AuNPs in both organs and an effect on the activity of the tested biomarkers, with the digestive gland being the primary target organ. While qRT-PCR expression levels raise some concerns regarding the effects of chronic AuNP exposure, at the tested concentration the particles did not cause significant oxidative damage. Further we could demonstrate a significant elimination of Au from the digestive track within a 7 day purification period, with excretion being an important pathway.

378
First evidences of PAMAM dendrimers internalization in microalgae and cyanobacteria: linkages with toxicity and oxidative stress
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Algae and cyanobacteria are ecologically relevant organisms which are at the base of aquatic food webs and have essential roles in nutrient cycling therefore being especially well suited to study possible ecological impacts of nanomaterials. We have applied a multi-end point approach to get insights into the toxic mechanisms of action of poly(amidoamine) (PAMAM) dendrimers on both *Chlamydomonas reindartii* (microalga) and *Anabaena* sp. PCC 7120 (cyanobacterium). Dendrimers are considered "perfect" polymers due to their symmetry, and have exponentially increasing applications in a variety of medical and technological fields. We chose generations G2, G3 and G4 native $–NH_2$ and NH-C-$(CH_2OH)_3$ (-OH) surface functionalized PAMAMs. In our approach, physicochemical properties of nanomaterials on relevant biological media were studied and linked with the biological information from the organisms. Special focus was posed on internalization and intracellular alterations induced by PAMAM dendrimers and their relationship with oxidative stress. We found that native $–NH_2$ PAMAM were toxic to both organisms in a generation-dependent manner; (-OH) surface functionalized PAMAM presented reduced toxicity with respect to their $–NH_2$ counterparts except the G4-OH congener which showed similar toxicity to that of G4-NH_2. The observed toxicity correlated with an increase in intracellular oxidative stress in both organisms, as identified by flow cytometry and confocal microscopy. However, the photosynthetic machinery seemed to remain unaffected which is in disagreement to results reported elsewhere [1,2]. Experiments performed with Alexa-Fluor488:PAMAM dendrimers revealed fast (10 min) internalization of dendrimers (in a generation dependent manner) with low retention time in cell wall/outer membranes, and a cytoplasmatic homogeneous distribution. However, chlorophyll---dendrimer/ROS colocalization was almost absent; emphasizing that the photosintetic machinery seemed to be unafected, and possibly it is not the source of oxidative stress. [1] Petit, A.-N., et al. Nanotoxicology, 2012. **6**(3): p. 315-326. [2]Petit, A.-N., et al. Aquatic Toxicology, 2010. **100**(2): p. 187-193. *Acknowledgement* - This study was supported by the Community of Madrid grants S-0505/AMB/0321and S-2009/AMB/1511and by the Spanish Ministry of Science grant CGL2010-15675/BOS and CTM2008-04239/TECNO and CTM2008-00311/TECNO.

379
Toxicity and accumulation of silver in Euglena gracilis upon exposure to silver nanoparticles and silver nitrate
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Engineered silver nanoparticles (AgNP) are potentially harmful to aquatic life. Little is known about AgNP interactions with algae. In the present study, the short-term effects and accumulation of cit-AgNP (citrate-coated) are examined in the alga *Euglena gracilis*, which has a pellicle on the surface instead of a cell wall, and is able to carry out endocytosis. The AgNP in exposure medium were characterized by dynamic light scattering. The level of AgNP dissolution was determined by ultracentrifugation and centrifugal-ultrafiltration. ICP-MS was used for metal analysis. Algae were exposed to various concentrations of cit-AgNP (0.1 – 40 μM) and to $AgNO_3$ (5 – 400 nM) as a source of Ag^+. After 1 and 2 hour, the photosynthetic activity was measured fluorometrically. To access the contribution of Ag^+ in the toxicity of cit-AgNP, cysteine was used as silver ligand. Biovolume were examined with 5 μM cit-AgNP and 50 nM $AgNO_3$ using cell counter, and morphology changes were also assessed by confocal microscopy. For accumulation experiments, *E. gracilis* were exposed to $AgNO_3$ (25 – 500 nM) and cit-AgNP (1 – 10 μM) for 1 hour and collected for ICP-MS measurments. The cit-AgNP remained stable during exposure in the medium, with a size range of 34 ~75 nm and a zeta-potential of -20 ~ -33 mV. The level of dissolved Ag^+ was 0.5% ~ 3.5%. Cit-AgNP and $AgNO_3$ lead to inhibition of photosynthesis with EC_{50} of 1858 nM for cit-AgNP and 85 nM for $AgNO_3$ based on total silver mass. Incresed biovolume and changes in morphology were oberserved upon exposure to both cit-AgNP and $AgNO_3$. Inhibitory and morphological effects of cit-AgNP were all prevented by cysteine. The accumulation of silver (Ag_{acc}) with $AgNO_3$ was linear when expressed as mol per cell, but non-linear when expressed as mol per Liter cell resulting from increased biovolume. In the case of cit-AgNP, the Ag_{acc} showed a non-linear increase. Based on dissolved Ag^+, Ag_{acc} was ~15 times higher with cit-AgNP compared to $AgNO_3$, which could corespond to 38~156 NP per cell, or to a dissolution of particles up to 44%. In sumary, the effects of cit-AgNP on *E. gracilis* photosnythesis, biovolume and morphology are mediated by dissolved Ag^+. The level of silver accumulation in *E. gracilis* upon AgNP exposure is high compared to $AgNO_3$, indicating sorption on to algal surfaces or cellular internalization.

380
Uptake of gold nanoparticles into Gammarus pulex from synthetic and natural waters
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Engineered nanoparticles (ENPs) are used in a range of product types, including personal care products, electronics and display technologies and other various applications. The increasing use of ENPs will result in increasing amounts of ENPs being released into the aquatic environment. Concerns have therefore been raised over the potential impacts of ENPs on aquatic organisms. Many studies of ENPs for ecotoxicological effects are based on acute or chronic tests following traditional test guidelines. However, most ecotoxicological risk investigations have ignored the fact that ENPs will aggregate to different degrees in different media types and the use of standard studies could either over or under estimate the real risk of ENPs. Our previous work demonstrated that the model gold nanoparticles (Au NPs) show different degrees of aggregation in both various ecotoxicological test media and in natural waters. In this study, we build upon our previous work and explore the implications of differences in aggregation in artificial media and natural waters for the uptake of ENPs by the fresh water shrimp, *Gammarus pulex*. The study used a set of model Au NPs with different properties and four different synthetic test waters and a variety of natural waters. In each medium, Au NPs showed different ranges of particle aggregation depending on the composition of the applied test media and natural water. To explore whether size explained the observed differences in uptake, we related mean particle sizes for each NP at the end of the uptake phase to measured uptake. The results indicate that size differences do not explain the observed differences in uptake and that other factors such as, the surface charge of nanoparticles and charge of the cell or gut membrane in a particular treatment may be influencing the uptake and internalization of the particles in test organisms. Therefore, for the risk assessment of nanoparticles, not only is important to select appropriate test media that reflect the uptake of NPs that will occur in natural systems. A better understanding of the interactions between the NP surface properties and environmental factors and uptake is required to understand how to extrapolate from effects observed under laboratory conditions to effects on the natural environment.

381
Toxicity of silver nanoparticles to a fish gill cell line: role of medium composition
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With the increasing application of silver nanoparticles (AgNP) in daily life, more attention is paid to the effects of AgNP on aquatic environments. In aqueous solutions, AgNP behaviour is affected by a variety of factors which lead to altered AgNP size and toxicity. This work aims to explore the effect of media composition on AgNP behaviour and toxicity to the RTgill-W1 cell line, which was derived from rainbow trout (*Oncorhynchus mykiss*) gill. Citrate coated AgNP (cit-AgNP, 20 nm, -30 mV) were used in this work. Cit-AgNP were added to three different exposure media: L15/ex, L15/ex w/o Cl and d-L15/ex. L15/ex is a modification of the original Leibovitz (L15) culture medium, which contains only salts, sodium

pyruvate and galactose. In this medium, cit-AgNP showed moderate agglomeration with sizes of 200-500 nm and zeta potential of -15 mV. L15/ex w/o Cl is a chloride free medium in which chloride is replaced with nitrate to avoid the formation of AgCl. Cit-AgNP heavily agglomerated in this medium (1000-1750 nm, -10 mV). Finally, to maintain cit-AgNP stable, a new medium, d-L15/ex, was developed by diluting L15/ex w/o Cl medium with water and adding NaCl. Cit-AgNP size ranged 40-100 nm in this medium and zeta potential was around -20 mV. The impact of cit-AgNP and $AgNO_3$ on cell viability was investigated. The extent of cit-AgNP toxicity differed between media with the toxicity rank order (highest to lowest) being L15/ex w/o Cl > L15/ex > d-L15/ex. When dissolved silver in cit-AgNP media was measured with ICP-MS (dissolved ratio: 0.4%-1.89%) and dose-response curves recalculated based on dissolved silver, the rank order changed to L15/ex w/o Cl > d-L15/ex > L15/ex. The low toxicity in L15/ex could be due to the high concentration of chloride, allowing AgCl formation. Furthermore, comparing cit-AgNP toxicity as function of dissolved silver and $AgNO_3$, $AgNO_3$ was less toxic in all media (10-40 fold). Besides, silver ligands could not prevent the cit-AgNP toxic effect to cells. Both indicated a particle-specific contribution to toxicity. In summary, our results showed that the toxicity of cit-AgNP is due to not only the dissolved silver, but also the particle-specific effect. Linked to the cit-AgNP behavior, deposition of cit-AgNP on cells might explain the higher toxicity of agglomerated cit-AgNP compared to that of suspended cit-AgNP.

Bioaccumulation processes and mechanisms: Implications for experimental assessments and modelling (I)

382

A tiered strategy for evaluating Bioaccumulation data in PBT assessments

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Tiered approaches to aquatic bioaccumulation (B) testing and also guidance on evaluating bioconcentration factor (BCF) data for organic chemicals generated using internationally recognised test guidelines (such as the Organisation for Economic Cooperation and Development [OECD] 305 [1]), have previously been proposed (e.g. Parkerton et al. [2] etc). However, there is a lack of guidance available for regulators and risk assessors for considering other bioaccumulation/bioconcentration (B) data, particularly when categorizing chemicals to be B or very B (vB) (i.e. BCF >2000 or >5000) in PBT assessments (i.e. persistent, bioaccumulative and toxic). Alternative approaches may be sufficient to determine whether a substance is clearly B or vB or if a substance is clearly not B (e.g. BCF < 500). However, some risk assessments or substance evaluations may not consider that sufficient information is available to perform the B assessment without having data from a full bioconcentration test. This means that substance evaluations and risk assessments may be relatively subjective depending on the understanding of the individual that is performing such evaluations. Furthermore, this may result in unnecessary testing requirements being requested by Regulatory Authorities. In addition, international regulations such as the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) regulation (EC No. 1907/2006) advocates the use of alternative (validated) approaches prior to the use of animal testing for data gap requirements. One difficult area of B assessments using alternative approaches is where there is some ambiguity as to whether the substance would cross the BCF 2000 threshold and be assigned as a bioaccumulative substance. These chemicals may be considered to be in the “grey area” of chemical bioaccumulation assessment and those within this category would require further testing in order to strengthen the B assessment. This paper summarises existing approaches which are available for assessing Bioaccumulation in fish and proposes a tiered testing approach to provide guidance to regulators and risk assessors on how to assess these data.

383

Octamethyltrisiloxane (L3): Assessment of biomagnification and toxicity through aquatic/dietary lab studies, fugacity ratios, and field data

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Lipophilic chemicals, such as the linear volatile methylsiloxanes (LVMS), are considered likely candidates for potential bioaccumulation in aquatic food webs, and sorb well to particles/surfaces, making sediments a key sink when performing environmental risk assessment evaluations. This project examined the laboratory and field data concerning the bioaccumulation and ecotoxicity of octamethyltrisiloxane (L3), an LVMS material. Experimental BCF and BMF results for L3 with various fish species are discussed in detail and then collectively used to model a TMF value for a Great Lakes aquatic foodweb. A fish BCF value of >5000 L/kg-ww exists for L3, a compound for which food uptake should dominate over water accumulation (log K_{ow} >6). To further evaluation of L3 bioaccumulation potential, a BMF study was conducted in accordance with OECD regulatory guidelines, consisting of a 35-day uptake and 30-day depuration phase and ^{14}C-L3 spiked into fish food (~500 μg/g) fed to juvenile rainbow trout (*Oncorhynchus mykiss*). In the dietary BMF study, an assimilation efficiency for food-to-gut transfer of 32% was determined, along with a steady-state kinetic BMF_K value of 0.26 and a lipid-adjusted kinetic BMF value ($BMF_{K(L)}$) of 0.86. All bioaccumulation data for L3 are then evaluated collectively as dimensionless fugacity ratio values in a weight-of-evidence approach, testing whether such ratios are collectively greater than (i.e., bioaccumulation) or less than (i.e., biodilution) 1.0. To evaluate environmental risk, chronic benthic toxicity no-observed effect concentrations (NOECs) for L3 are compared to field sediment concentrations using a probabilistic risk assessment (PRA) approach, which allows the risk assessor to include stochastic properties of both exposure and response. The PRA method examined field sediment L3 concentrations at a variety of global locations and compared the 95th centile concentration to the 5th centile chronic toxicity NOEC value from benthic organism studies. The analysis revealed no overlap of exposure and toxicity at these levels and a factor greater than 10,000-fold between the respective exposure and NOEC centile levels. Collectively, these analyses indicate an absence of bioaccumulation and risk to aquatic species posed from this linear methylsiloxane.

384

How well can we model bioconcentration factor (BCF) from dietary accumulation or biomagnification factor (DAF / dietary BMF)?

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Bioaccumulation potential is a fundamental indicator of ecotoxicological behavior and ecological risk of chemicals. It is commonly measured by the aqueous exposure-based bioconcentration factors (BCF). However, BCF does not reflect a chemical’s bioaccumulative potential via dietary uptake; furthermore, its measurement can be operationally challenging for chemicals with low aqueous solubility. Consequently, it is desirable to measure the bioaccumulation potential of chemicals through dietary exposure, which would produce dietary accumulation factors (DAF) or dietary biomagnification factor (dietary BMF). Although dietary bioaccumulation protocol has been incorporated in recent standard chemical testing guidelines, regulatory statutes are predominantly established based on BCFs. In order to bridge the gap between dietary bioaccumulation studies and the regulatory need for BCF values, this study examined the basis and the extent to which we can estimate BCF from dietary bioaccumulation experiments. Fish dietary BMF data (n≈600) corresponding to a total of 184 weakly polar to apolar organic chemicals were reviewed and extracted from primary literature. BCF was predicted from the dietary bioaccumulation data using measured or derived total depuration rate constant (k_T) and predicted respiratory uptake rate constant (k_1) based on various k_1 models. Analysis suggested that k_T determined from dietary and aqueous exposure experiments were statistically identical. This implied that k_T can be determined from dietary exposure and may be applied in the bioconcentration scenario. Using dietary accumulation data and an accurate respiratory uptake rate constant (k_1) model, BCF can be estimated to within ±1 log unit (root mean square of errors = 0.64) of the mean experimental values. Furthermore, the estimated BCF is independent of the dietary dosage applied for up to 4 orders of magnitude in the exposure concentration, and this eliminated the need for case-by-case normalization. Although the examined BMF-BCF estimation protocol is generally successful, further improvement in our ability to predict k_1, greater confidence in experimental BCF values, and more dietary studies on organic chemicals with lower hydrophobicity are critical if a tighter convergence between dietary BMF and BCF is desirable.

385

The environmental relevance of active transport inhibition

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The uptake of organic chemicals into the cell as the frist step of toxicokinetics is believed to be governed by physico-chemical partitioning. Recently, there is increasing evidence that this purely physico-chemical process is superimposed by active transport, resulting in an energy-dependent efflux of chemicals, which reduces the bioavailability and bioaccumulation of chemicals in cells. This transport mechanism may be inhibited by chemosensitisers. These are mainly xenobiotic substances which are commonly present in the environment, such as pharmaceuticals, pesticides, fragrances and surfactants. Available data on active transport inhibition has been collected and analysed with regard to the environmental relevance of this mechanism. On the one hand, membrane and cell-based in-vitro assays predict effect concentrations of active transport inhibitors in the range of several mg/L. However, they are often conducted using unrealistically high exposure concentrations and single or binary mixtures, only. Moreover, artificial active transport protein overexpression may lead to the underestimation of toxicity in such test systems. On the other hand, in-vivo tests have produced lower effect concentrations. Those still exceed environmental

aqueous concentrations by several orders of magnitude. Despite this, exposure of organisms to environmental mixtures could be proven to enhance the bioaccumulation of active transport substrates, thus suggesting the presence of chemosensitisers in environmental samples causing observable effects in organisms. In conclusion, from the data present it is difficult to come to a decision about the environmental relevance of chemosensitisation. While the comparison of effect concentrations with environmental data suggest that no effects should occur in the environment, there is evidence for the opposite stemming from the exposure of aqueous organisms to complex environmental mixtures. As this evidence is few, more focus needs to be put on such complex and more realistic exposure scenarios.

386

The power of analogy: predicting the properties and bioaccumulation behaviour of per- and polyfluorinated alkyl substances (PFASs) using their fatty acid counterparts.

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Poly- and perfluorinated alkyl substances (PFASs) are analytically challenging, with physicochemical properties that are difficult to measure. Their bioaccumulation behavior differs from that of neutral hydrophobic organic compounds in important ways. They accumulate preferentially in the blood and liver, rather than in lipids, and there is growing evidence that their strong associations with proteins may be key controllers of their elimination half-lives and bioaccumulation potential. In this talk we will consider how the analogy between PFASs and fatty acids can be used to help predict their physicochemical properties and interactions with proteins, and how this information can be integrated into new models for PFAS bioaccumulation. We have surveyed the PFAS and fatty acid literature in order to identify parallels in their properties and behavior that will allow us to use existing fatty acid research to help fill persistent data gaps surrounding PFAS properties. We explore these properties and protein interactions using a mechanistic bioconcentration model we recently developed for perfluorinated alkyl acids in fish. By incorporating the interactions of PFASs with three key protein types—serum albumin, membrane transport proteins, and fatty acid binding proteins—into a physiologically-based pharmacokinetic model, we explore the way these interactions control the tissue distribution and elimination kinetics of different PFAS structures. Previous attempts to correlate PFAS bioconcentration factors with BSA-water distribution coefficients have met with only limited success. A model structure than can integrate multiple protein interactions is required. This indicates that attempts to develop new QSARs for these chemicals will need to somehow incorporate these multiple interactions.

387

Screening for Low Aquatic Bioaccumulation: Prediction Confidence and Quantitative Weight of Evidence with the BCF Waiving Scheme

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Aquatic bioconcentration factors (BCF) are critical in PBT and risk assessment of chemicals under REACH. High costs and consumption of more than 100 experimental animals per standard BCF study (OECD 305) call for filters based on quantitative structure-activity relationships (QSARs). The BCF waiving scheme allows to identify substances with low aquatic bioaccumulation (nonB, BCF < 2000) based on physico-chemical properties related to media-specific exposures and bioavailability (hydrophobicity, air/water partitioning, biodegradability, hydrolysis, ionisation). The nonB compounds cannot classify as PBT and their bioconcentration testing may be waived because of low concern with regard to the B criterion. The initial BCF waiving scheme [1] has been improved with an extended dataset of reliable BCF data for 998 compounds and complies with the OECD principles for valid QSARs. It performs with 100% sensitivity (no false negatives) and 60% efficacy (waiving potential). Specific bioaccumulative chemicals are excluded from the applicability domain. Reliability is based on (i) the number of physico-chemical property criteria triggered by query compounds, (ii) the distance of property estimates from thresholds and (iii) the structural similarity with known nonB and B substances. Prediction confidence of the BCF waiving scheme relies on applicability domain coverage defined by structural features (chemical classes, atom centred fragments (ACF)) and on the reliability rating. Quantitative Weight-of-Evidence (WoE) for low aquatic bioaccumulation is inferred from a Bayesian combination of BCF waiving scheme predictions, weighted by their reliability, and consistency with other available information including estimates from independent QSARs. Bayesian statistics provide quantitative measures of the WoE and inform users about the reliability of the classifications as either nonB (BCF definitively < 2000) or "unknown" (BCF may be >2000). *Acknowledgement* - This work was supported by the EU 6th Framework Integrated Project OSIRIS (contract no. GOCE-ET-2007-037017). [1] Nendza M, Herbst T. 2011. Screening for low aquatic bioaccumulation (2): Physico-chemical constraints. SAR QSAR Environ. Res. 22, 351-364.

Mechanistic effect modeling - beyond concentration response and constant environments (I)

388

Toxicokinetics and toxicodynamics of imidacloprid in six aquatic macroinvertebrates: model calibration and evaluation of predictions

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The systemic insecticide imidacloprid (IMI) is a nicotinoid insecticide that has been used for insect pest control since many years. Non-target aquatic organisms could potentially be exposed to imidacloprid via input from spray drift, leaching, or runoff after spraying. We used raw data on the survival over time of *Asellus aquaticus, Caenis horaria, Cloeon dipterum, Chaoborus obscuripes, Gammarus pulex* and *Plea minutissima* from acute and chronic laboratory tests on the toxicity of IMI (Roessink et al., 2013) to estimate the parameters of two TKTD models, namely the reduced versions of the stochastic death (SD) and the individual tolerance (IT) model (Jager et al., 2011). The more sensitive species (*C.horaria, C.dipterum* and *P. minutissima*) stand out with higher killing rates (kk, SD model) and lower internal median threshold values (α, IT model), respectively. The interpretation of the parameter values suggests that the reported higher toxicity of IMI towards these species is related to an increased internal sensitivity and not to an increased uptake or accumulation. Furtheron, model predictions of the survival of the six species under chronic exposure were done using the parameter sets of the fitted models for the acute experiments, and vice versa. The goodness-of-fit of the predictions was evaluated by calculating the ratios between the log-likelihood values of the predictions and the fits, respectively. For *A.aquaticus, C.obscuripes, C.horaria* and *C.dipterum* the log-likelihood values for model predictions were not deviating by more than a factor of 1.5 from the model fit, hence indicating a quite good quality of model predictions. Model predictions for *G.pulex* deviated strongly from the evaluation data sets. Reasons for the latter observation are not clear yet. This study demonstrates that it is possible to use data from standard toxicity testing of aquatic invertebrates for the parameterisation of TKTD models. The resulting parameter values were useful for an improved understanding of the reasons for the high toxicity of imidacloprid to some aquatic macroinvertebrates. The quality of the parameterised TKTD models with respect to predicitons of survial under new exposure situations was evaluated to be surprinsingly good for most species. It appears that it is possible to use TKTD model parameterisations based on standard toxicity testing of a range of species for extrapolations of the survival under more complex exposure scenarios.

389

A SYSTEMS BIOLOGY APPROACH REVEALS A NOVEL CALCIUM-DEPENDENT MECHANISM FOR BASAL TOXICITY IN DAPHNIA MAGNA

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The release of an increasingly larger number of anthropogenic chemicals into the environment and the interaction between pollution, land use and climate change, represents a formidable challenge in ecological risk assessment. An important component in this process is a consideration of the potential toxicity of chemicals to ecologically relevant organisms. Rapid, predictive, mechanism based and cost effective approaches for ecological risk assessment of chemicals are urgently needed to preserve the integrity of the natural environment. Recently, adverse outcome pathways (AOPs) have been suggested as a framework for risk assessment to link the molecular mechanisms of chemical action through to the adverse effects (toxicity) on an organism. Quantitative structure activity relationships (QSARs) provide an established alternative to traditional toxicity tests for the identification of toxic chemical. However, these computational approaches generally do not provide a mechanistic link between physical chemical features and the predicted adverse outcome. To overcome these limitations, our group has recently proposed to integrate QSAR analysis approaches with the molecular response of an organism and relevant biological endpoints into a comprehensive strategy to provide structure guided adverse outcome pathway prediction. Here, we address the question of identifying toxicity mechanisms of a panel of diverse chemicals in the crustacean *Daphnia magna*, a species of great environmental relevance. The approach we use relies on computational models that link compound Physical Chemical Features (PCFs) with molecular response pathways defined by sub-lethal transcriptional signatures predictive of organism toxicity. Model predictions and experimental validation support the hypothesis that intracellular calcium release triggered by lipophilic chemicals may be one of the initiating events that underlie basal toxicity of these compounds. We have described the first example of an experimentally validated integration of traditional QSAR analysis, functional genomics and ecotoxicology in a quantitative and predictive computational framework. This approach led to formulating the first working model explaining the molecular basis

of the basal toxicity of lipophilic chemicals, a finding that is bound to have broad implications across all areas of toxicology.

390
An energy-based model to evaluate toxicity of an endocrine disruptor: a case study with tributyltin and the pond snail
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Endocrine disruptors are compounds that adversely alter functions of the endocrine system with consequences for population stability and recruitment. Therefore, the European Union allows marketing and use of chemicals only if they do not exert endocrine disrupting properties. Aquatic molluscs are recognised as organisms sensitive to such chemicals and the development of standardised mollusc toxicity tests is under way. However, these tests do not aim at an integrated assessment of the effects of chemicals on all endpoints over time. To demonstrate that such an integrated assessment can aid the risk assessment of endocrine disruptors, we present a case study with tributyltin (TBT), a chemical known for its endocrine disrupting properties in some molluscs. We combined experimental and toxicokinetic-toxicodynamic (TKTD) modelling approaches to evaluate toxicity of TBT in the freshwater gastropod species *Lymnaea stagnalis*. Effects of TBT on life-cycle traits were interpreted within the framework of dynamic energy budget (DEB) theory. To obtain data suitable for model calibration, we exposed adult and juvenile snails (which became adults during the test) to TBT in two independent toxicity tests over a part of the life cycle. Snails were fed with fresh lettuce. Additionally, snail eggs were exposed to TBT concentrations to assess effects of the compound on the embryonic development. A TKTD model called DEBkiss was used to analyse effects of TBT on survival, growth, and reproduction of snails simultaneously. TBT affected survival of juveniles only, and growth and reproduction of juveniles and adults. The complete set of data from the two partial life-cycle toxicity tests was well described by DEBkiss. The overall pattern of effects on growth and reproduction strongly suggests that assimilation of energy from food is decreased by TBT. Relevant output for ecological risk assessment (ERA) are no-effect concentrations (NECs) for survival and sub-lethal effects of 49.5 and 1386 ng Sn/L, respectively. Furthermore, TBT induced a decrease of hatching success to less than 10%, but only at the highest concentrations. The lack of effects on hatching time and hatchling size indicates that TBT affects an aspect of the feeding process that is specific for exogenous feeding on lettuce. We demonstrate that coupling a relatively simple test design with TKTD modelling approaches offers a great potential for the improvement of current ERA for endocrine disruptors in invertebrates.

391
Combined effects of an endocrine disrupting chemical, elevated climatic temperature and inbreeding on sexual development and population viability in fish
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Sex determination in many fish is temperature dependent; therefore the sex-related effects of endocrine disrupting chemicals (EDCs) could be exacerbated by future changes in climate. Small inbred fish populations, which are susceptible to inbreeding and stochastic variation in population size and sex ratio are likely to be particularly at risk from environmental impacts on sex determination. In a laboratory-based empirical study we investigated the masculinising effects of the EDC clotrimazole, in combination with elevated temperature. These combined effects were studied with inbred and outbred zebrafish (*Danio rerio*). Experimental results were used to parameterise a population viability analysis model to assess the population consequences of masculinisation. Inbred (F_{IT}=n+0.25) and outbred (F_{IT}=n) wild-type zebrafish families (n=20) were exposed in the laboratory from 40-100 days post fertilisation (dpf) to clotrimazole (0, 1.7, 8µg l^{-1}) at 28°C and an elevated water temperature (33°C). Sex ratios were skewed towards males in inbred and outbred fish following exposure to 8µg clotrimazole l^{-1} at 28°C (82% and 60% males, respectively) and skews were greater at 33°C (97% and 83% males, respectively). There was also significant male-skew in inbreds (82%), but not outbreds following low-level clotrimazole exposure (1.7mg l^{-1}), in combination with elevated temperature. Population viability analysis showed that probability of extinction (PE) was largely insensitive to these sex ratio skews. Population growth rate (r), however, declined sharply in response to male-skews with a lower decline threshold in inbreds (>80%) compared with outbred fish (>90%), highlighting a risk for inbreds based on our experimental results. Including compounding inbreeding depression on juvenile survivorship further reduced r, with minimum viable population size increasing from 500-10000 adults. In summary all three factors, clotrimazole exposure, temperature elevation and inbreeding, skewed sex ratios significantly towards males and their effects were additive, having the potential to impact fish populations. High fecundity in zebrafish (r-strategist) offers a buffer against environmental skewing of sex ratios, but short generation times, leading to rapid inbreeding and inbreeding depression in survivorship can erode this buffering capacity in small, isolated populations.

392
Extent and time-dependency of synergistic effects in D. magna after pulsed exposure to azole and pyrethroid pesticides
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Two of the most commonly used classes of pesticides in agriculture are azole fungicides and pyrethroid insecticides, which constitute a large proportion of the total fungicide and insecticide usage. Several studies including both laboratory and field studies have reported synergistic interactions between azole fungicides and pyrethroid insecticides towards both insects and aquatic invertebrates. Azole fungicides are believed to enhance the toxicity of pyrethroids by inhibiting the cytochrome P450 monooxygenases responsible for xenobiotic detoxification and possibly also by increasing the uptake rate of the pyrethroids. The purpose of this study was to investigate the extent and time-dependency of azole induced synergism on acute toxic effects of alpha-cypermethrin in the freshwater invertebrate *Daphnia magna*. Since cytochrome P450 inhibition by the azoles is known to be fast and reversible, we hypothesise that the persistence of the synergistic potential after exposure to an azole pulse is primarily determined by the elimination kinetics of the azoles. Hypothesis were tested by exposing *D.magna* to a 18 hours pulse of one of the azoles propiconazole or prochloraz followed by a six hours pulse of alpha-cypermethrin. The dependency of the synergistic potential on the time between pulses was analysed by varying the time between the two pesticide pulses and simultaneously monitoring alpha-cypermethrin induced effects on mobility. GC-ECD was used for complementary measurements of internal azole concentrations - linking the time course of internal concentrations to the synergising potentials. For the treatments where the daphnids were exposed to the alpha-cypermethrin pulse immediately after the azole pulse, propiconazole and prochloraz caused a 7 and 13-fold increase in effect, respectively. Observed synergism decreased with increasing time between the pulses. The estimated first-order elimination rate constant of the synergistic potential of propiconazole was 0.31 h^{-1} (95 % CI: 0.08-0.54), indicating that for more than 13h between pulses no difference in immobility between daphnids treated or non-treated with azoles can be observed anymore. The elimination rate constant for the internal concentrations of propiconazole was 0.23 h^{-1} (95 % CI: 0.21-0.26) (Fig 2b). We therefore conclude that toxicokinetics of the azoles seem to be determining for the persistence of the synergistic potential towards alpha-cypermethrin in *D. magna*.

393
Predicting population recovery from chemical exposure: the influence of the environmental scenario
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Recovery of populations is an important attribute in ecological risk assessment of chemicals. Recovery in the field does not depend on the chemical's properties and the exposure profile only, but it is strongly linked to important environmental variables as well. Yet, these are only marginally considered in risk assessment due to the limitation of the experimental methods in grappling with such complexity. Here, we use individual-based modelling to investigate how the environmental scenario affects the recovery of *Daphnia magna* populations from chemical exposure. Simulation experiments were performed for chemicals with lethality levels ranging from 40% to 90 % at different food and temperature conditions. The same toxicity levels were then tested in combination with species interactions including predation or competition. We showed that recovery of populations strongly depended on the environmental scenario. This dependency was reflected in the highly heterogeneous population responses to the same chemical when the environmental context is changed. We conclude that it is necessary to define the ecological scenario when assessing the recovery of populations in ecological risk assessment of chemicals. Validated population models will greatly assist with such an investigation.

Policy assessment in an integrated systems perspective: indicators and targets to ensure operating within safe planetary boundaries (I)

394
The UNEP/SETAC Life Cycle Initiative: Mainstreaming Life Cycle Approaches for Sustainable Consumption and Production
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In order to put life cycle thinking into effective practice, UNEP and SETAC launched the Life Cycle Initiative (Initiative) in 2002. The first phase (2002-2007) focused on establishing the Initiative as a global focal point of life cycle (LC) related knowledge and activities, and building a community of practitioners and stakeholders. During phase 2 (2007-2012), the Initiative became more participative with increasing involvement from global stakeholders. Common understanding and agreement were achieved on questions like Life Cycle Assessment (LCA) databases. Results of surveys and consultations in 2011 showed that whilst LC capacities have increased, especially in Asia and Latin America, efforts are still needed to support progress in many countries which have limited LC based experience and knowledge, and weak regulations. Such issues are being addressed in phase 3 (2012-2017). Finally, more and better communication is required to engage with current and potential decision makers towards better informed decisions on more sustainable products (UNEP/SETAC, 2012). Building on the achievements and outputs from phases 1 and 2, the phase 3 vision agreed is a world where LC approaches are mainstreamed. Activities focus on creating the enabling conditions to (a) enhance the global consensus and relevance of existing and emerging LC methodologies and data management; (b) expand capabilities worldwide and make LC approaches operational for organizations; and (c) communicate current LC knowledge to influence and partner with stakeholders. The adoption of the Ten-Year Framework of Programmes (10YFP) on sustainable consumption and production (SCP) and its five initial programmes in 2012 in Rio+20 (UNEP, 2012), provides a good opportunity for the Initiative to support countries strengthening the delivery of LC approaches. The abstract provides a brief overview of past activities of the Initiative and first insights in the plans for phase 3 and introduces the possibilities for interlinkages with the programmes adopted under the 10YFP in support of the development of SCP plans worldwide. UNEP (2012). A 10-year framework of programmes on sustainable consumption and production patterns. A/CONF.216/5 UNEP/SETAC (2012). Greening the economy through life cycle thinking. Paris, 2012

395
Multidimensional assessment of natural resources in LCA for resource policy support
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Availability of natural resources and access to them are two fundamental conditions for the well-being of human societies. The sustainable use of natural resources and the access to them has become a high policy priority, both from an environmental and sustainability point of view but also from an economic and business oriented perspective. Several impact assessment methods exist for assessing resources in LCA; there isn't consensus in the scientific community on how to assess this aspect across sustainability assessment methodologies. Based on literautre review, a scheme for a multidimensional approach to resource has been depicted in order to support resource policies. The identified research needs for improving the impact assessment of resources regard: enlarging the number and typologies of resources modeled; developing a common framework that allow resource comparison and options for substitution; considering the effect of scarcity in natural environment and the ecological role of resources; assessing the effects of recycling and anthropogenic stocks in the overall evaluation of availability and taking into account socio-economic and strategic issues such as security of supply and criticality. A list of aspects that should be taken into account in a comprehensive impact assessment of resources has been compiled. These aspects can be clustered into five groups: 1. aspects related to resource properties (e.g. thermodynamic loss due to the resource extraction; resource renewability; involvement in biogeochemical cycles; role as natural habitat (e.g. freshwater/forest) and in natural habitats); 2. Aspects related to resource availability (e.g. biophysical availability; resource scarcity, resource criticality, which includes also the geo-political constrains); 3. technological constrains/opportunities (e.g., recyclability and substitutability) and 4. environmental impacts (e.g. damages on habitats, landscape, etc.).

396
Geopolitical Supply Risk Assessment: The case of France
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The supply riks of resources is one of the priorities in the political agenda of the European Commission. The list of critical raw materials (CRM) for the European Union (EU) was identified under the framework of the EU Raw Materials Initiative, with the aim of helping decision makers to take relevant action to avoid potential supply disruption and to secure a sustainable supply of resources. Mineral resource impacts are typically quantified in Life Cycle Assesment (LCA) through assessing depletion. However, the use of mineral resource use beyond its rate of replacement as an impact assessment metric has several problems and it has been widely debated within the LCA community. Resource use evaluation for materials such as metals should go beyond depletion. Life cycle impact assessments (LCIA) need to address the supply riks issue, which does include not only the geological element but also socio-economic and other geopolitical factors that affect accessibility. This requires a new perspective of LCIA that would rather give emphasis to a broader dimension of availability. In this work we mainly aim at looking how the issue of resource criticality assessment could be brought under the general framework of life cycle sustainability assessment (LCSA). A method to assess geopolitical related supply risk is proposed and also applied for the case of selected strategic resources for France. The result suggests that REE, Sb, Nb, and Be are the top four resources which are subjected to high supply risk. The largest portions of the risk in the case of REE for example, are mainly contributed by China. China is by far the leading global supplier of REEs which results in having higher market concentration, one of the determinant supply risk factors in the proposed method. Besides having the largest global market share, China is also one of the most important trading partners of France for REE supply, which accounts for around 90 of the total import shares. In the case of Nb the supply risk value is determined by the large import reliance on South Africa, Germany and the UK . The geopolitical supply risks of In, RE, PGM, Co, U and Se are relatively low. This could be in most cases due to their wide global market distribution: i.e. in the case of Uranium the HHI value is 0.19 compared with the highly concentrated mining for REE which has an HHI value of 0.91.

397
Full integration of LCA with other assessment tools – new application areas and harmonized modelling approaches
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Commonly, life cycle assessment (LCA), input-output analysis (IOA) and mass flow analysis (MFA) are seen as separate assessment tools each with specific application areas. Recent and ongoing EU 6th and 7th framework projects are creating and integrating several different national accounts enabling for a full integration of the above mentioned assessment tools. The following projects together have led to the creation of the, to date, most detailed and complete set of integrated model for LCA, IO analysis and MFA: Exiopol, FORWAST, CREEA and DESIRE. The integrated model, which is called the exiobase, is a global multi-regional hybrid IO database which is based on fully balanced monetary, mass and energy accounts (supply use tables). The current database includes the following extensions: economic value added, and physical accounting for resources, emissions, land use, and water consumption. Further, work is ongoing for integrating with more detailed LCA databases such as ecoinvent, adding social issues to the extensions, and to separate land use change activities from existing industries enabling for explicit modelling of indirect land use changes (iLUC). The database has several application areas for use as an assessment tool for policy development at different levels of scopes like product, corporate, project, program and policy impact assessment, at different levels of organization from individual company to government/intergovernmental, and at different geographical scales from local to global. Some of the advantages of integrating the different assessment tools and data are that much overlap and inconsistency are avoided. Further, the use of a common classification and terminology also adds to increasing consistency. Having one integrated framework enables for using any new data together with existing data in a complete database rather than having several separate incomplete and inconsistent models, databases and assessment tools. With the database environmental footprints of nations can be calculated using exactly the same models and data as corporate environmental footprints of enterprises (such as environmental profit & loss accounts) as well as product specific LCAs. With an integrated assessment tool the advantages of several separate tools and datasets are merged, and coherence between international and national policy development with lower levels such as projects, programmes, products and enterprise is facilitated.

398
Integrating LCA, Scenario Modelling and Multi-criteria Decision Analysis for Sustainable Policy-Making in the Energy Sector
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In the light of climate change, concerns about security of energy supply and limited non-renewable resources, new policies for the energy sector are urgently required. Policy-making in this field is affected by various environmental (e.g. greenhouse gas emissions), economic (e.g. investment cost) and social (e.g. impacts on human health) aspects, i.e. by all three pillars of sustainability. In order to evaluate different policies in the energy sector (e.g. nuclear phase-out) concerning their impact on a sustainable development, scenarios are developed. To quantify scenarios, economic partial equilibrium (PE) models are used to find the cost-optimal energy technology mixes that satisfy exogenously defined energy service demands under the relevant political boundary conditions.
Complementarily, methods assisting policy-makers to make sound decisions in consideration of multiple sustainability criteria are applied. Multi-criteria decision analysis (MCDA) is a decision-making tool that aims at the comparison of different options taking into account a variety of sustainability criteria on the one hand and subjective stakeholder preferences on the other hand. MCDA integrates all three pillars of sustainability and applies the life-cycle approach in the environmental, economic, and social areas. In the present work, the two approaches, PE models and

MCDA, are combined with the purpose of assisting policy-makers in evaluating the role of Carbon Capture and Storage (CCS) in future Swiss energy policy. This work uses the Swiss MARKAL PE model for the quantification of three exemplary scenarios. The three cost-optimal energy technology mixes are calculated for a scenario without climate goals, one with climate goals but no CCS available, and one with climate goals and CCS available. Based on these three cost-optimal energy technology mixes in 2035, on the 15 calculated economic, social and LCA-based environmental indicators and on the subjective weighting profile of the decision-maker, the sustainability ranking of three scenarios is established using MCDA. Depending on the weighting, the three scenarios are ranked differently, revealing the advantages and disadvantages of the three policies under assessment. The two methods are complementary, combining the system-wide perspective of PE models and the broad suite of criteria considered in MCDA. This work can be taken further towards an integrated method including multi-criteria assessment in the optimisation process.

399

Evaluating the sustainability of recycling packaging materials combining LCA and Fuzzy Set Theory

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In a study for the Swiss retailer association the possibility to extend the actual recycling to various materials has been examined. The Swiss retailer association wanted to know which recycling and logistics system (e.g. separate or combined collection) would be the best from an overall point of view. For some criteria's and materials quantitative results from LCA and LCC were available for other materials only estimations. For a sustainable system other criteria's are also important like satisfaction and acceptance of consumers or future markets. For these only qualitative knowledge and estimations exist. To combine and evaluate the different type of information Fuzzy Set Theory (FST) was used because this mathematical framework permits to combine the available quantitative results from LCA and LCC with the qualitative or linguistic expert judgements. Floating transitions typically for environment can be handled and the uncertainty of estimations are taken into account. To model and evaluated this complex system in a transparent and comprehensible way a FST model has been developed together with experts from different fields like logistics, recycling technologies, ecology and economy. The result of this study was twofold. First of all it gave a good decision base for the retailers as well as for the municipalities and national administration to there policy of sustainable, future collection and recycling systems. Second it could be shown that this methodological approach is not only useful but very powerful for decision support of complex systems. The important advantages of this method are: • representation of qualitative expert knowledge with linguistic terms • bringing together quantitative and qualitative data and knowledge from different fields like ecology, economy and societal as well as technical aspects • nonlinear relations can be handled • floating transitions which are typical for environment can be handled • Fuzzy logic can deal with more than one 'right' judgement typical for human reasoning • doing all this in a transparent way. So we gained the experience that for complex decisions FST will be one of the efficient ways for decision making according to the statement of Lofti Zadeh: "In almost every case you can built the same product without FST, but fuzzy is faster and cheaper."

Environmental OMICs: high-throughput strategies to decipher mechanism of response to stressors (II)

400

Proteomic response of L. rubellus, the red earthworm, to metal stress under different soil temperatures

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Proteomic approaches bear the possibility to study the response to changing environmental conditions on the organismic level. This may lead to a deeper understanding of stress-induced cellular processes compared to classical approaches. In the present study, *L. rubellus*, the red earthworm, was exposed to Cadmium under different soil temperatures with the perspective to unravel putative synergistic effects between environmental pollution and the impact of global warming. The availability of a sequenced genome, a peptide-, EST- and RNA-Seq database for this species renders *L. rubellus* a highly suitable model organism to explore the impact of environmental stressors also on the proteome level. Protein extracts from controls and Cd exposed earthworms (15°C and 20°C) were separated according to their isoelecteric point. Gels were stained with colloidal coomassie blue and digitized for statistical analysis. Significantly changed protein spots were excised from the gels and analyzed on a mass spectrometer obtaining peptide mass fingerprints (PMF) which were combined with tandem mass spectra to search the *L. rubellus* peptide and EST databases to identify the protein spots using Mascot software. Statistical analysis revealed 69 significantly changed protein spots in the treatment groups out of 549 detected spots on the fusion image. 45 protein spots revealed significant ID hits, whereas for 25% of protein spots no significant hits were obtained from the database search. However, all significant hits reveal 3 groups of proteins with similar expression patterns in the heat map. Significant protein hits were assigned to functional categories such as Cytoskeletal, Energy metabolism, Calcium Signaling, Vesicle-, Ion- and Oxygen Transport, Redox System, Lysosomal and Thrombolytic proteins. Cd exposure at 20°C clearly showed a different expression pattern compared to the control group and is, moreover, also differing from the Cd-exposure at 15°C. In fact, 64% of significant changes differ within the two temperature groups, whereas only 36% show a similar behavior in the treatments compared to the controls. The proteomic response to Cadmium stress seems to draw a distinction under different habitat temperatures. Weather this effect has a severe negative impact on the earthworm, or might rather reflect the induction of detoxification mechanisms, is still to be elucidated.

401

Stressor-induced proteome alterations in zebrafish: widespread artefacts or a universal stress response?

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Proteomics are being increasingly applied in ecotoxicology on the premise that the identification of changes in the expression of specific proteins and/or protein groups occurring in response to a certain chemical would allow elucidation of the underlying molecular pathways leading to an adverse effect. This in turn could promote the development of focused testing strategies for specific groups of toxicants. Unfortunately, the global proteome characterization techniques applied most often, both gel-based and gel-free, do not allow a complete coverage of all proteins expressed at a given moment, measuring instead only a fraction of those being most abundant in the sample. Nonetheless, such analyses do detect expression changes for some proteins, based on which certain conclusions are drawn regarding the cellular processes affected. The somewhat disturbing feature of such studies is the fact that a few protein groups seem to respond regardless of the nature of stressor applied. To investigate the issue more closely, we analyzed the studies that looked into the changes induced by various physical, chemical and biological stressors in the proteome of zebrafish, a model organism popular in ecotoxicology. Our meta-analysis highlights several protein groups, including heat shock proteins, enzymes involved in energy metabolism and cytoskeletal proteins, to be most frequently identified as responding to a stressor in zebrafish, regardless of tissue or stressor analyzed. With this we demonstrate that the "hit parade" situation previously described for mammalian studies seems to be a common phenomenon readily spreading to other species and research fields. The question that arises next is whether these proteins are truly responding and thus may potentially represent a "footprint" of a general stress response, or if this situation is a consequence of current technical limitations to global proteomics analysis. We present data evidence, ideas and hypotheses in support of both options and would like to promote an open dialog on the topic. We suggest that the results of any differential proteomics experiment performed with zebrafish (or any other species) should be interpreted keeping in mind the list of the most frequent responders identified. Careful consideration of the reliability and significance of observed changes is necessary in order to prevent the proliferation of false positive linkages between the stressor and the cellular functions it perturbs.

402

Deciphering the major egg yolk proteins from the amphipod Gammarus fossarum by proteogenomics: key lessons regarding prediction of the functions of ecotoxicology-relevant proteins.

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Recently, we proposed an approach for quickly identifying proteins of the freshwater crustacean *Gammarus fossarum*, an ecologically and ecotoxicology relevant by the alliance of genomic and proteomic (*i.e.* proteogenomic). We have generated a large transcriptome dataset using Illumina-Solexa pyrosequencing and in parallel acquired massive shotgun proteomics data. By now, we were able to identify a total of 1,873 proteins that have been certified by tandem mass spectrometry. These proteins were functionally annotated on the basis of sequence similarities. But for a new model organism with important phylogenetic distance compared to available sequenced models, automatic pipelines as shortcut for functional annotation can leads to inaccurate predictions due to quite different evolutionary scenari. Here, we aimed to provide accurate functional annotation to proteins members of the large lipid transfer protein (LLTP) family, characterized by an intense functional diversification. We specifically identify vitellogenin (VTG), proteins involved in the formation of egg-yolk in our model species. Using label-free proteomic quantitation, analysis on embryos and ovaries sampled at different stages of their development resulted to the identification of 378 and 573 proteins respectively. Next, we focused our attention on the proteome dynamics for identification of putative VTG candidates proteins with accumulation profiles over the oogenesis cycle were chosen. On the over way around, as egg yolk protein are consumed during embryogenesis, providing resources for organogenesis, proteins with pattern of consummation profiles over the embryos development were chosen. Finally, the cross comparison between the proteins isolated from the relevant temporal variations and the ones previously classified as female specific, led to a list of 8 major egg yolk proteins for *G. fossarum*, all part of the LLTP family. For the two most abundant ones, functional predictions annoted then as clottable proteins, *via* decapods homology queries. Our study pointed out the necessity that for any new model organism, data from omics studies must be confirmed in terms of organism-specific function before being considered in terms of ecotoxicological perspectives. For the sentinel species *G. fossarum*, our multi-omics results identified the gene products specifically involved into its reproductive function. This should result in interesting biomarker of endocrine disruptor exposure.

403

Physiological and omic analysis reveal cryptic effects of low environmentally-relevant levels of chemical stressors on terrestrial plant species

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Terrestrial plant communities can be confronted with xenobiotic combinations of bioactive molecules, degradation products, and adjuvants that constitute chemical challenges potentially affecting plant growth and fitness. Such complex challenges involving residual contamination and mixtures of pollutants are difficult to assess. The model plant *Arabidopsis thaliana* was subjected to chemical stress involving the herbicide glyphosate, the fungicide tebuconazole, the glyphosate degradation product aminomethylphosphonic acid (AMPA), and the atrazine degradation product hydroxyatrazine, which had been detected and quantified in soils of field margins in an agriculturally intensive region. Field margin contamination levels were shown to have significant effects on plant growth and metabolism despite low levels of individual components and the presence of less toxic pesticide-derived metabolites. Metabolomic and molecular analysis of these physiological effects demonstrated that pesticide degradation products AMPA and hydroxyatrazine elicited significant plant responses, thus indicating underlying mechanisms of perception and transduction. Such mechanisms were also in line with observed interactions, whether positive or negative, between the effects of AMPA and hydroxyatrazine and the effects of bioactive xenobiotics (glyphosate and tebuconazole). Furthermore, the metabolic and molecular perturbations induced by low levels of xenobiotics and associated degradation products were shown to affect processes (carbon metabolism, amino acid metabolism, hormone balance, stress-response mechanisms) that are likely to determine environmental stress sensitivity. These effects of low levels of edaphic contaminants were also investigated in *Lolium perenne*, a plant species that is commonly found in field margins and used in vegetated filter strips at the edge of agricultural fields. Combination of physiological and omic analysis revealed that residual environmentally-relevant levels of chemical stressors induced significant modifications of carbon-, nitrogen- and stress-related pathways at the transcriptomic and metabolomic levels. Detailed knowledge of these fine and subtle perturbations and regulations in terrestrial plant species, under conditions of single or multiple pollution, is necessary for the complete understanding and predictive modelling of environmental risks associated with residual contamination.

404

Health impact assessment of emerging contaminants tested at low environmentally relevant doses individually and in mixture using genomic and metabolomics approach

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There is increasing evidence that contaminants of emerging concern (CEC) pose health risk to animals and humans. However, insufficient information is available about the mechanisms by which these compounds cause adverse physiological and pathological effects. Previous studies demonstrated the presence of a number of pollutants in the Oldman River, Canada. This study was carried out to investigate the effects of a number of more abundant contaminants at low environmentally relevant concentrations, individually and in mixture, using genomic and metabolomics approach. To test the hypothesis that CECs cause disruption of health in fish, we performed controlled laboratory experiments in which goldfish and fathead minnows were exposed to the same concentrations of chemicals detected in the river system, individually and as mixtures. Multiple end points, including expression of various genes involved in gonadal development and differentiation were measured. Using ^{1}H-NMR metabolomics as a tool, we also measured the concentrations of multiple metabolites in the liver, gonad and brain tissue, and evaluated net metabolic dysregulation due to exposure. The results suggest significant dysregulation of amino acid, lipid, energy, carbohydrate, nucleotide and cofactor/vitamin metabolism. In the liver and testis, the effects of contaminant mixture were found to be significantly different from those of individual compounds tested. We also exposed fathead minnows to the same contaminants, individually and in mixture, and performed microarray analysis. The "Omics" data collectively provide information on cellular response to contaminants and identified novel biological endpoints. The findings will facilitate development of better tools to evaluate risk through mechanism-based cell and tissue response, and assess susceptibility of fish exposed to environmental contaminants. In particular, metabolomics and genomics study of low dose response to individual and mixture of chemicals allowed us to make a more accurate assessment of adverse health impact in fish. Together with our previous field data, the present results provide a framework for better understanding of ecological consequences of exposure to CECs in fish and other vertebrates. *Acknowledgement* - The authors thank NSERC of Canada to provide funding.

405

High-throughput nanospray metabolomics to identify mixtures of chemical stressors and their effects in fish exposed to wastewater effluents

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Many biologically active contaminants are present in final effluents of wastewater treatment work (WwTWs) and can bioaccumulate as complex mixtures in fish. The use of LC-MS-based nontargeted chemical profiling and metabolomics analyses is a very promising tool to assess both chemical exposure and its associated metabolite effects in the same sample. Current metabolite profiling methods do not detect trace levels of endocrine signalling compounds present in extracts of tissues or plasma due to interference from highly abundant components. We developed novel nanoflow ultraperfomance liquid chromatography-nanoelectrospray ionization-time-of-flight mass spectrometry, and improved solid phase sample extraction techniques in order to profile low abundant components in the metabolome. The aim of this study was to investigate the chemical mixtures accumulating in fish, and the associated changes in the tissue metabolomes to determine the potential deleterious physiological effects arising from effluent exposure. Sexually mature roach were exposed for 15 days to either a treated effluent from a WwTWs or to clean water. Extracts of plasma and tissues (gonads, kidney, liver and gill) from effluent-exposed and control fish were compared using nontargeted chemical and metabolite profiling methods. Identified contaminants accumulating in tissues of effluent-exposed fish included endocrine disruptors and mixtures of many pharmaceuticals such as nonsteroidal anti-inflammatory drugs, selective serotonin re-uptake inhibitors, benzodiazepines, antipsychotics, anticonvulsants, beta blockers, fibrates, and anticoagulants. Metabolite profiling revealed disturbances in eicosanoid, steroid, serotonin, bile acid, carnitine and sphingosine pathways. Effluent exposure resulted in widespread reduction of prostaglandins in many tissues and these mediators are important in ion transport, immune function and reproduction. In addition, reduction in androgen and increases in serotonin metabolites were observed, indicating potential effects on reproductive and neurological endpoints. Metabolite disruption was apparent although the plasma concentrations of the likely causative contaminants were 3-1000 fold below human therapeutic levels indicating possible mixture effects. The use of this nontargeted ‘Omics’ approach could be extremely informative for ecotoxicological investigations on the health effects and associated contaminant mixtures in fish exposed to wastewater effluents.

Soil Biodiversity and Ecotoxicology (II)

406

What do we need to improve ecological relevance in soil risk assessment? State of the art and perspective for Collembola

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The current tier 1 risk assessment of plant protection products (PPP) for soil organisms according to the EU Regulation (EC) 1107/2009 involves a test on earthworm reproduction and one on nitrifying microorganisms. An additional Collembola reproduction test is required under specific conditions. Higher tier studies should take into account a more realistic substrate and/or exposure regime, or communities, e.g. in (semi-) field studies. We introduce potential intermediate and higher tiers involving Collembola, in particular a modelling approach which takes into account the vertical distribution of PPP and Collembola. We discuss potentials and limitations of different approaches which had been presented and discussed on a workshop at RWTH Aachen University on October 22-23, 2013. Emphasis is given on the identification of data requirements and gaps that would be necessary to better meet existing protection goals for soils.

407

Prediction of effects of sea level rise on soil ecosystems
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The increase of global mean temperature is raising serious concerns all over the world due to its potential negative effects on the planet such as droughts and melting of glaciers and ice caps. These events are originating the rising of the seawater level and, thus, facilitating its intrusion into the terrestrial systems. The estimations for 2100 predict that such rise will be between 47 and 190 cm. Expected impacts on soil compartment include floodings, water intrusions and subsequent use of saltwater for irrigation, with unknown effects on soil ecosystems and their inhabitants. Integrated in a broader research project, the present study aimed at evaluating the effects of salinisation on soil ecosystems due to sea level rise. The reproduction and mortality of three standard soil invertebrates (the springtail *Folsomia candida*, the enchytraeid *Enchytraeus crypticus* and the predatory mite *Hypoaspis aculeifer*) in standard artificial OECD soil irrigated with serial dilutions of seawater or concentrations of salt (NaCl), was evaluated according to standardised guidelines. Results showed no effects of all the seawater dilutions used in adult mortality. However, for reproduction, an increased sensitivity was observed in the following order: *H. aculeifer*

408
Deriving a terrestrial PNEC for silver: use of research findings in risk assessment
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The presence of silver (Ag) in the terrestrial environment and the potential risks that may be associated with this metal are currently receiving increased interest. A research programme was undertaken by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) into the toxicity and bioavailability of silver in soils funded by the European Precious Metals Federation. The programme was undertaken to assess the influence of leaching, aging and soil properties on the behaviour, bioavailability and ecotoxicity of silver, primarily to allow this knowledge to be incorporated in regulatory risk assessment e.g. under the EU REACH regulation. This presentation will describe the interpretation of the findings of the CSIRO programme in order to deliver a terrestrial risk assessment for the uses of silver under REACH. The fundamentals of this process have been performed for other metals, such as copper and nickel. However, this is the first time that site-specific bioavailability-based assessment has been undertaken for silver.

409
Characterization of ecotoxicity and phytotoxicity of a cyanobacterial extract containing microcystins under realistic environmental concentrations and in a soil-plant system
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The occurrence of harmful cyanobacterial blooms in surface waters is often accompanied by a production of variety of cyanotoxins that represent a hazard for human and animal health. Microcystins (MCs) are the most common cyanotoxins and may be expected wherever blooms of cyanobacteria occur in surface water with more than 80 variants have been characterized. Microcystin-LR (MC-LR) is generally recognized as being the most studied variant due to its high toxicity and frequent, and concentrations in surface waters often exceed the World Health Organization (WHO) advisory level of 1 μg L^{-1}. More recently, phytotoxic effects of MCs after irrigation were evidenced. But, to our knowledge, there is no study reporting experiments performed in realistic exposition: environmental concentrations and using soil-plant system approach. Here, we used these conditions to characterize both the ecotoxicity on soil microorganisms and the phytotoxicity on the tomato plant *Solanum lycopersicun* var. MicroTom of a toxic crude extract of *Microcystis aeruginosa* (PCC 7820) at environmental concentrations usually reported in the surface waters (0, 20, 50, and 100 μg equivalent MC-LR L^{-1}). Soil enzymatic activities (arylsulfatase, β-D-glucosidase, phosphatase and urease), nitrification potential and growth of seedling were studied. The effect of MCs on bacterial nitrification communities was evaluated by RT-PCR using primers for genes linked to Ammonia-Oxidizing Achaea (AOA) and Ammonia-Oxidizing Bacteria (AOB). In the presence of MCs at the high concentration (100 μg equiv. MC-LR L^{-1}), the germination of seeds was not inhibited and the global soil microbial activities are not altered. However, the activity and abundance of nitrifying bacteria are significantly affected at the lower concentration (5 μg equiv. MC-LR L^{-1}). In addition, the growth of tomato seedlings increased significantly at this concentration.

410
Earthworm reproduction studies: Is the growth of body weight during the range finding test a good indicator for successful reproduction?
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Earthworms are the standard test organisms for testing possible side-effects on soil organisms caused by plant protection products (EC REGULATION No 1107/2009) or chemicals (ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7c: Endpoint specific guidance (2012)). For the environmental risk assessment, the endpoints LC_{50} (acute toxicity: test concentration which results in 50% mortality) and NOEC (sublethal toxicity: No-Observed-Effect-Concentration) are required. Normally, these endpoints are delivered by the OECD TG 207 for the acute toxicity during 14 days of exposure and OECD TG 222 for sublethal toxicity, i.e. reproduction rates of earthworms exposed for 28 days. Generally, the relevance of these endpoints is based on the careful selection of test concentrations which is especially critical for the NOEC. Therefore, the EC_x-approach is recommended for the substitution of NOEC by EC_{10}/EC_{20}. For the selection of the test concentrations in the definitive test with 5-6 concentrations, usually a range-finding test is performed. In the case of acute toxicity (OECD TG 207), the design of the range-finding test is similar to the definitive test with the endpoint mortality, but with a reduced number of concentrations and replicates. In the case of the sublethal test (OECD TG 222), the respective range-finding is an acute test with an exposure of 14 days and the determinination of body weight loss as sublethal endpoint. From our point of view, the critical question should be asked which relation can be expected between body weight loss and reproduction in earthworms. Earthworm body weight and reproduction might be positively or negatively correlated due to allocation of energy investments dependent on the actual biotic and abiotic conditions for an individual worm. Therefore, we present a meta-analysis of earthworm acute toxicity and earthworm reproduction studies in order to answer the question to what extend the body weight growth is a good indicator for successful reproduction and propose amendments for the next revision of the OECD TG 222.

411
Guideline values of lead in soils: a new soil-ecotoxicity approach
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Soil contamination with lead is known worldwide and can cause adverse effects on human health and the environment. Mobility and availability of Pb in terrestrial environment is strongly controlled by the soil properties. An usual key tool for the analysis of bioavailability and toxicity of metal(loid)s is the use of bioassays, which contribute to assess the ecological risk and help as reference to declare contaminated soils. The aim of this work is to study the influence of different soil properties and constituents on the solubility of lead in laboratory-contaminated soils, to propose guideline values of this element based on toxicity bioassays. We used seven soils with contrasting properties (H1-H7), with different polluted levels of Pb selected from Andalusia proposed values (0-500-1000-2000-4000-8000 mg kg^{-1}) and performed three bioassays: Root Elongation Toxicity Test (*L. sativa*), Microtox® Test (*V. fischeri*) and Soil Respiration Test. According to our results, there was a significant correlation between bioassays; while the main variables influencing toxicity in all soil types were pHW, PbW and PbT. In the non carbonated soils, the toxicity was also significantly related to OC, P and CEC. Furthermore, concentrations of soluble lead show significant differences between samples in all polluted levels, being carbonate-rich soils (H1, H2 and H3) the samples with lower Pb solubility. Thresholds obtained (NOEC, EC10 and EC50) indicate that soils with the lowest phytotoxic effect were those rich in carbonate content (H2 and H3); with reductions below 50% for the maximum contamination level (8000 mg kg^{-1}). Followed by the soils rich in organic carbon (H1 and H5), being the no carbonated and low organic content soils (H4, H6, H7), the ones with highest toxicity response. Out of the three bioassays carried out was *L. sativa* test which presented the highest sensitivity to toxicity. Andalusian Government proposes guidelines values based exclusively on the pH, however, our results show that these values could be overestimated when referring to carbonated and organic-rich soils and underestimated in other soil types. Lead toxicity is strongly influenced by soil properties; therefore soil guideline values of Pb should be set at different levels depending on soil properties, to avoid over- and under-estimations and to optimise the efficiency and profitability in the application of generic environmental quality standards.

Community and ecosystem ecotoxicology (II)

412
Effects of abiotic factors on structure and abundances of freshwater macrofauna in ditches along bulb fields
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A large area in the Netherlands is used for flower cultivation. Maintaining balance between high yields and low risk to the environment is a main purpose of the environmental policy in the Netherlands. The amount of pesticides used in bulb crops has decreased in the last 20 years, however despite this fact the amount of chemicals applied in bulb fields remains relatively high. Ditches therefore are continuously affected by chemical contamination that results in adverse effects on non-target aquatic biota. Current research aimed to assess the effects of pesticides in combination with abiotic factors on the structure and abundances of aquatic macrofauna communities in ditches next to bulb fields. Field work was based in flower growing region of the Netherlands. The area is intensively used for flower bulb growing, mainly hyacinths, lilies, daffodils and tulips. There are several patches of pastures and grasslands. Macrofauna samples in the field ditches and water chemistry data were collected in the period April-November (2011-2012). Field data was assessed by means of multivariate analysis. Abundances of sensitive insect taxa Odonata and Diptera were larger at the nature reserve sites. Whereas high numbers of the insect Hemiptera, mollusks Basommatophora, Heterostropha and the annelid Haplotaxida were associated with the agricultural area. Abiotic factors, along with pesticides, contributed significantly to variations in the macrofauna community structure. In the natural aquatic environment organisms are affected by multiple stressors. Abiotic factors should be considered when evaluating the effects of pesticides on aquatic biota. \n

413
A glimpse in the black box – Ecotoxicological impacts on the Yangtze Three Gorges Reservoir, China
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The creation of the Three Gorges Reservoir caused the flooding of former urban, industrial and agricultural areas. Consequently, substantial amounts of organic and inorganic pollutants were released into the reservoir. Beyond contaminants and nutrients enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. In addition the dam reduced the flow velocity of the river for about one magnitude, changing this section from river-like to lake-like. This has a serious influence on the dilution of the pollutants that enter the water body, and also an impact on the sedimention rate of supended particles. Sediment functions as a sink for a large variety of organic contaminants from which they can again be remobilized. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediment onto agriculturally used areas along the reservoirs shore. Although a large number of studies have been performed along the Yangtze River, the TGR section remains a black box, little iluminated only by a couple of studies mainly focusing on the chemical analysis of pollutants in the compartments water and sediment. Thus, it is reasonable to ask (a) what is the current pollution status of this newly created ecosystem, which is a source of food and water for millions of people, (b) is the local fauna, e.g, fish species affected and (c) which would be the responsible priority pollutants. In order to assess (i) possible links between molecular/biochemical responses and ecologically relevant effects, and (ii) if ecotoxicological effects might be related to adverse effects in fish from the field, sediment samples and fish were collected at different locations in the Three Gorges Reservoir as well as its catchment area and analyzed using a weight-of-evidence (WOE) approach with several lines of evidence. So far sediment and fish samples have been taken at major cities as well as feeder rivers and their estuaries along the Yangtze Three Gorges Reservoir in order to characterize the state of contamination and the potential risk for human and wildlife. The monitoring strategy should help to observe the water body's quality and to serve as a basis to initiate if necessary counteractive measures. **Keywords:** Yangtze, sediment, fish, monitoring

414
Hexabromocyclododecane affects plankton communities and benthic-pelagic coupling in an experimental ecosystem
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Hexabromocyclododecane (HBCDD) is an additive brominated flame retardant used mainly in expanded and extruded polystyrene foam for insulation and construction, textiles and electronic appliances. HBCDD was recently added to Annex A of the Stockholm Convention on Persistent Organic Pollutants due to its persistent, bioaccumulative and toxic properties. As for most chemical substances, evidence for HBCDD's ecotoxicity comes mainly from standardised single species tests. However, little is known about its effects on coastal species, and even less on ecosystem effects. We investigated the effects of a range of HBCDD concentrations on experimental ecosystems over an 8 month period. The ecosystems were assembled in 1000 L mesocosms from naturally-occurring components of shallow coastal Baltic Sea areas. HBCDD was added spiked into a phytoplankton suspension at the start of the experiment and a range of structural and functional endpoints were measured. Of relevance to this presentation are: phytoplankton and zooplankton community structure; nutrient concentrations in the overlying water; biomass of *Macoma balthica*, an infaunal filter- and deposit feeder; HBCDD concentration in water, sediment and *M. balthica*. Changes in plankton community structure were analysed using correspondence analysis. Generalized Additive Mixed Models (GAMMs) were used to analyse the time-dependent relationships between chlorophyll *a*, nutrients and HBCDD dose. Structural equation modelling (SEM) was used to test the hypothesis that HBCDD can affect ecosystem structure and function, and two SEMs were constructed; pelagic system interactions and benthic-pelagic coupling. Increasing HBCDD dose had a negative relationship on the biomass of large *Macoma balthica,* apparently due to higher mortality of large *M. balthica* at high HBCDD exposure. This in turn appears to have led to lower bioturbation rates and related recirculation of nitrogen to the water column (i.e. a decreased benthic-pelagic coupling) at these higher doses. Changes in pelagic communities were then observed, first in the phytoplankton and a few weeks later in the zooplankton communities. These results demonstrate the complexity of the ecosystem response to HBCDD, incorporating many indirect effects in the system over time. Such responses can only be quantified and understood by using realistic experimental set-ups, and by including knowledge of system-specific ecological interactions when assessing ecotoxicity.

415
Pesticide mixture toxicity and effects on benthic invertebrates and algae in agricultural streams – field and laboratory studies
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Within the framework of a national monitoring program, we collected water samples from 4 streams in small agricultural catchments to study pesticide mixture toxicity. Mixture toxicity was assessed by calculating ∑Toxic units and the Swedish pesticide toxicity Index (PTI) for algae, Daphnia and fish. In the lab growth inhibition tests were run with green algae and Daphnia to test for effects at 0.1, 1, 10, and 100 times the observed ∑TU in the field. Tests were run with reconstituted water samples containing observed peak pesticide concentrations (∑TU).In the field, samples of benthic invertebrates and diatoms were collected in the 4 streams on a monthly basis (spring to fall) to study community effects. More than 10 pesticides were detected in ≥63% of the 902 samples collected during 2002–2010, with 90-percentiles ranging 21–29 pesticides. The average number of pesticides in a single water sample ranged from 8.8±3.7 to 17.8±7.4, while the maximum was 41 pesticides. Our data show marked peaks in estimated toxicity (PTI and ∑TU), mainly occurring between May and July. Despite the high number of pesticides detected, mixture toxicity (∑TU) was frequently dominated by only one or a few compounds. Algal growth was inhibited at concentrations corresponding to between 1 and10-times the ∑TU observed in the streams. Also for daphnids effects were found at 10–100- times the observed ∑TU in the streams. These results show that negative effects occur at concentrations. PLS showed that the diversity of benthic diatoms was negatively correlated to our estimates of toxicity (i.e. $\sum TU_{algae}$ and PTI), suggesting lower diversity at higher levels of toxicity. Effects on the macroinvertebrate community were mainly explained by in-stream physical and chemical conditions. Our results show that mixture toxicity is a common feature in agricultural streams. Our results also suggest that diatom diversity is negatively related to pesticide toxicity and that the well established metrics developed for eutrophication and/or organic loading do not detect these effects.

416
Pollution Induced Community Sensitization (PICS): exploring the relevance of the conceptual coun-terpart of Pollution Induced Community Tolerance (PICT)
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Ecotoxicological experiments and assessments usually build on the assumption of a continuous exposure to one single toxicant. Validated concepts for assessing the ecological consequences of pulsed exposures, e.g. from spray or run-off events are still largely missing. Central for generating a hypothesis on the expected overall joint ecological effects after sequential exposure to toxicants is the phenomenon of Pollution-Induced Community Tolerance (PICT). PICT postulates that pollution

events from compounds with a similar ecological mode of action lead to an increased community tolerance. Here we present its conceptual counterpart, Pollution-Induced Community Sensitization (PICS). PICS builds on the assumption that an organism's susceptibility towards dissimilarly acting chemicals is negatively correlated: an organism that is highly susceptible towards a particular substance is assumed to be less sensitive to a compound with a completely different ecological mode of action, and vice versa. Experimental results from a study of sequential pulses of antibiotics thought to be targeting predominantly gram-negative (Colistin), respectively gram-positive bacteria (Novobiocin) in microbial biofilms indeed indicate an elevated toxicity after pulsed exposure, in comparison to a continuous exposure. These results will be presented and discussed, also making use of modeling studies that employ species-sensitivity distributions.

417

Competition determines toxicant -effect, -recovery and -culmination of successive toxicant pulses

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Pesticides applied in agriculture can affect the structure and function of non-target populations at lower doses and for longer timespans than predicted by the current risk assessment frameworks. Obviously the sensitivity of communities in real-world ecosystems are strongly determined by the environmental context. In detail we identified that the (i) strength of the toxicological effect is governed by the magnitude of intraspecific competition; the (ii) duration of recovery is governed by the magnitude of interspecific competition; when (iii) successive exposure is present an enhancement of trans-population effects culminate in a strong final effect. The underlying processes are experimentally identified and reconstructed using a simulation model. We conclude that repeated toxicant pulse of populations that are challenged with interspecific competition may result in a multigenerational culmination of low-dose effects.

Environmental biodegradation rates and pathways: Dependence on environmental conditions

418

How a new builder in automatic dishwashing detergent became ready biodegradable in the U.S.: Widespread microbial adaptation

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L-GLDA (Glutamatediacetate) is a builder that was introduced as a phosphate replacement in a United States (U.S.) automatic dishwashing detergent (ADW) in early 2010. Prior to ADW use, L-GLDA failed multiple biodegradation screening tests (OECD 301B) in the U.S. and was not considered ready biodegradable in this geography. However, results from a more realistic continuous activated sludge treatment test (OECD 303A) predicted that a microbial population pre-exposed to L-GLDA would extensively degrade it after a lag period. Other evidence indicating L-GLDA's potential to biodegrade included positive ready biodegradability test results obtained in The Netherlands and the isolation of a bacterium that could utilize L-GLDA as a sole nitrogen, carbon and energy source [1]. The market introduction of this new ADW in the U.S. and the subsequent widespread distribution of L-GLDA in wastewater offered a unique opportunity to monitor for potential microbial adaptation in the field. After launch of the ADW, a series of OECD 301B tests were conducted using inoculum collected from multiple WWTPs in two U.S. regions. Within several months, WWTP inoculums that had previously produced negative results, measuring 9-21% CO_2 production, demonstrated that L-GLDA was "ready biodegradable" (65-100% CO_2). Inoculum from additional WWTPs also showed positive ready test results and a decrease in the measured lag period (< 10% CO_2) was observed as distribution of the ADW grew, indicating an increase in the field population of competent degraders. To our knowledge, this is the first systematic study showing how a new chemical, that consistently failed ready biodegradation tests prior to market penetration became ready biodegradable across a wide geographical region following its introduction in a commercial product. The ready test is required under most regulatory schemes for the registration of a new chemical and to demonstrate a lack of persistence. However the use of pre-exposed inoculum is strictly prohibited in some regions. This work demonstrates that when pre-exposure occurs under realistic conditions, laboratory tests can accurately predict ultimate real world behavior indicating the importance of considering potential adaptation in regulatory assessments.

419

Using biodegradation for the removal of sulfonamides

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It has been shown, that the presence of antibiotics in the environment, contribute to the formation and spread of resistance genes among bacterial strains (Baran et al. 2011). Especially waste water treatment plants (WWTP) are proposed to be a hotspot for the emergence of resistant bacterial strains (Gao et al. 2012) and one of the major sources for the input of micropollutants into the environment (Abegglen and Siegrist 2012). Sulfonamide antibiotics are the second most used antibiotics worldwide in human and in veterinary medicine with a release of ~20,000 tons $year^{-1}$ (Baran et al. 2011). Nonetheless the biodegradation pathway of sulfonamide antibiotics is not understood. In addition, several studies reported insufficient sulfonamide removalrates by conventional sewage treatment(Gros et al. 2010). Even tertiary treatments like chlorination and ultraviolet radiation for disinfection, seem to have limited effect on the removal of sulfonamides (Batt et al. 2007). *Microbacterium* sp. BR1 was the the first isolated strain with the proven capability to partially mineralize Sulfamethoxazole (SMX) (Bouju et al. 2012). Degradation studies were carried out with this strain and revealed that the initial attack of SMX is carried out by the biological rare *ipso*-hydroxylation and that 3-amino-5-methylisoxazole is released as a stable metabolite, whereas the aniline moiety was mineralized by *M*. BR1. It could be shown, that *M*. BR1 is not only able to degrade SMX, but also five other tested sulfonamide antibiotics (Sulfadiazine, Sulfadimehoxine, Sulfamethizole and Sulfamethazine) and the herbicide Asulam. It is likely, that much more sulfonamides with a similar structure can be degraded by *M*. BR1, which makes it a suitable candidate for the biological treatment of sulfonamides. We were able to concentrate the protein responsible for the initial sulfonamide attack and identified as a $FMNH_2$ dependend monooxygenase. The results presented in this work lead to a better understanding of the biodegradation of sulfonamides. It is initiated by the biologically rare *ipso*-substitution and releases a predictable metabolite, which might be used as a biomarker together with a screening for the monooxygenase genes. Based on those results operational parameters of WWTP might be adapted to optimize sulfonamide degradation during the biological treatment, or at least clarify if the sulfonamide removal in WWTPs occurs due to biodegradation or other processess.

420

Fate and metabolism of Tetrabromobisphenol A in soil under different environmental conditions

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Tetrabromobisphenol A (TBBPA) is one of the most commonly used flame retardants and has become an environmental contaminant worldwide. However the fate and metabolites of TBBPA in the environment are still unclear. Using ring-^{14}C-labelled tetrabromobisphenol A (TBBPA), the transformation of TBBPA in soil under oxic and anoxic conditions and in water saturated soil with plant (rice and reed) growth was studied. While debromination was the predominant pathway for TBBPA degradation in anoxic soil, aerobic degradation of TBBPA in soil resulted in large amounts of mineralization (CO_2), single benzene ring metabolites, and *O*-methylation metabolites. Four primary metabolic pathways are proposed for aerobic degradation of TBBPA in soil, i.e., oxidative skeletal rearrangements, *O*-methylation, type II ipso-substitution, and reductive debromination. The presence of wetland plants (rice and reed) stimulated degradation of TBBPA and accumulation of mono- and di-*O*-methyl-TBBPA in soil. Substantial bound residues were formed during both aerobic and anaerobic degradation of TBBPA in soil. When soil redox potential altered from anoxic into oxic state, almost half of the anoxically formed bound residues were released as TBBPA and lower brominated BPAs, which were then persistent during oxic incubation. Our results provide detailed information about metabolites of TBBPA in soil and the first evidence for release of bound residues during alteration of the redox environment.

421

pH-dependent Biotransformation of Ionizable Organic Micropollutants in Activated Sludge

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A variety of organic micropollutants originating from households and industry enter wastewater treatment plants (WWTPs) where they are partially removed through conventional activated sludge treatment. The operating conditions as well as the removal efficiencies for micropollutants vary between different WWTPs. Since, on average, every second micropollutant contains an ionizable functional group and the pH of WWTPs can range between 6 and 8, the chemical species actually present in the WWTP may range from fully uncharged to fully charged. Therefore, it is not suprising that the removal efficiency of ionizable compounds has been observed to be pH-dependent. Besides pH-dependent sorption, pH-dependent biotransformation might be a plausible explanation for this findings. The goal of this project was to elucidate the mechanisms responsible for the pH-dependence of biotransformation of ionizable organic micropollutants in activated sludge. Biotransformation experiments were carried out in laboratory batch reactors seeded with activated sludge at pH 6, 7 and 8. A mixture of 20 compounds, consisting of 16 basic, two acidic and two neutral compounds, was spiked into the reactors each at a starting concentration of 100 μg/L. Samples were taken at 13 time points over a period of four days and analyzed with liquid chromatography coupled to high-resolution tandem massspectrometry. Additional control experiments that accounted for losses through sorption and abiotic processes were run in parallel. Concentration time series were fitted and corrected for sorption and abiotic processes, yielding rate constants that represent microbial biotransformation only. pH-dependence of the biotransformation rate constant was observed for most tested compounds. Basic compounds generally showed increasing rate constants with increasing pH, whereas the acidic test compounds showed the opposite trend. The underlying reasoning for this observation is the following: The fraction of neutral species is depending on the pH of the medium and the pKa of the compound. The uptake of uncharged molecules into the cell is assumed to be much faster than that of charged species, whereas biotransformation in the cell is expected to be similar at different external pHs due to cell homeostasis. This suggests that biotransformation is faster at pH levels where the uncharged species of a specific compound dominates. This hypothesis will be quantitatively tested based on the results from our study.

422

Potential for biodegradation of crude oil in the arctic marine environment

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Suggested presence of oil and gas resources in the seabed off Greenland has along with climate change induced reductions in sea ice created interest in exploration of the area. Exploratory drillings are performed at up to 1000 m depth which has led to concern for deep sea oil spills. In deep sea oil spills biodegradation is a key factor for oil removal however the capacity for oil biodegradation in the arctic marine environment is not elucidated. To investigate this, a microcosm study was performed using water samples from 150 m depth in the Disko Bay, Greenland. Indigenous marine microorganisms were exposed to crude oil in concentrations of 0 mg, 2.5 mg, 5 mg, and 10 mg × l^{-1}. Microcosms were incubated for 71 days and samples were withdrawn at six sampling times in total. Bulk water phase was extracted separately from remaining water and oil phase. Through oil fingerprinting analysis by gas chromatography mass spectrometry (GC-MS) weathering effects on the oil phase were evaluated. Extensive biodegradation was evident for saturated hydrocarbons and alkyltoluenes at all oil concentrations. Shorter chain saturated hydrocarbons were preferentially degraded compared to longer chains and the highest oil concentration provided the lowest degradation rate. Limited biodegradation of PAHs and their alkylated homologs was seen for 2-3-ringed PAHs and some alkylated homologs in the 2.5 and 5 mg × l^{-1} microcosms, while more extensive biodegradation was found for the same compounds in the 10 mg microcosms. These results were, however, affected by large replicate variation. Changes in oil fingerprint over time were similar in all concentration microcosms and extensive biodegradation was seen in 10 mg microcosms suggesting that oxygen was not a limiting factor in the experimental set up. The separation of water and oil phases in the present study further demonstrated that PAHs and their alkylated homologs were highly affected by dissolution, with an inversely proportional relation between dissolution and number of aromatic rings as well as degree of methylation. Results obtained from the present study indicated that should a deep sea oil spill occur in the Disko Bay saturated hydrocarbon fraction and alkyltoluene fraction of crude oil will be rapidly biodegraded by indigenous marine microorganisms. PAHs and their alkylated homologs may be biodegraded to a lesser extent. Keywords: Crude oil, biodegradation, arctic, oil spill

423

Modelling the fate of petroleum hydrocarbons in bioengineered piles in the Antarctic

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Antarctic and sub-Antarctic soils are commonly contaminated with petroleum hydrocarbons. Biopiles can be used effectively to treat hydrocarbon contamination but their efficacy at low temperatures has, hitherto been uncertain. Here, we describe a dynamic multi-media model of petroleum hydrocarbon behaviour in biopile soils in which the temperature dependency of partitioning and degradation is included. The activation energy for degradation was derived by fitting the Arrhenius equation to hydrocarbon concentration data from temperature-controlled laboratory experiments using diesel- and crude oil- contaminated soil from Kerguelen Island. The model was then applied to a remediation biopile system containing soil contaminated with Special Antarctic Blend (SAB) diesel fuel at Casey Station, Antarctica. The model was able to describe temporal changes in total petroleum hydrocarbon concentration very well, provided temperature adjustments for degradation and partitioning were included. The quality of model fits for individual hydrocarbon fractions was variable but generally acceptable (disparity between measured and predicted concentrations < factor 2 for all fractions and in all biopiles). For most fractions, biodegradation was predicted to be the dominant loss mechanism, except for the lighter aliphatic fractions for which volatilisation was predicted to be most important. Despite the fact that losses during the winter are expected to be negligible for most fractions, summertime losses were significant, resulting in concentrations between 48 and 60 % of initial concentrations one year after the start of treatment.

Identification and prioritisation of hazardous emerging pollutants (II)

424

Changes of Accumulation Profiles from PBDEs to Brominated and Chlorinated Alternatives in Marine Mammals from South China Sea

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The present study investigated the composition profiles and levels of polybrominated diphenyl ethers (PBDEs) and five PBDE alternatives in the blubber of two species of marine mammals, Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) from the South China Sea. Despite the fact that PBDEs were the most predominant brominated flame retardants in the samples analyzed, decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), bis- (2-ethylhexyl) -tetrabromophthalate (TBPH), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and Dechlorane Plus (DP) were all detected in both cetacean species. In addition, significant increasing temporal shifting trends of Deca-BDE to DBDPE, Octa-BDE to BTBPE, and Deca-BDE to DP were observed in porpoise samples between 2003 to 2012 and dolphin samples between 2003 and 2011. These patterns may be attributed to the replacement of PBDEs by alternative halogenated flame retardants (HFRs) and the increasing usage of these alternatives following the restriction/voluntary withdrawal of the production and use of PBDE commercial mixtures. Our findings suggest that the study region may be a source of contamination by PBDE alternative flame retardants due to the high detection frequencies and levels of these compounds in marine mammals.

425

Environmental fate and effects of new generation flame retardants

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There is a growing need to substitute brominated flame retardants (BFRs) with alternative halogen-free flame retardants (HFFRs). Many of these HFFRs are already being marketed, although their environmental behavior and toxicological properties are known to only a limited extent and their potential impact on the environment cannot yet be properly assessed. Therefore the aim of the present project was to study the environmental fate and effects of a selection of halogen free flame retardants that are currently replacing brominated flame retardants in polymers. To this purpose we: 1. Published a rewiew on the publicly available PBT data of HFFRs, identifying large data gaps and inconsistent observations on the properties of individual compounds. 2. Quantified the aerobic biodegradation of HFFRs and observed that only TPP was mineralised rapidly enough to be classified as readily biodegradable. The rates of primary biodegradation of the OPFRs varied, with TPP, RDP and DOPO being removed to undetectable levels in less than 14 days. In contrast, no significant removal was observed for BDP. 3. Assessed the aquatic ecotoxicity of HFFRs. MPP, MHO, ZHS and ZS showed no effect at their saturated water concentration (Sw) and APP, ALPI and DOPO had a low toxicity, suggesting that these compounds are not hazardous. ATO had a moderate toxicity (EC_{50} = 3.0 mg L^{-1}) and TPP was highly toxic to *D. magna* (EC_{50} = 0.55 mg L^{-1}). ATH and BDP caused limited mortality at Sw (26 and 25% respectively) and have low solubilities (< 10 mg L^{-1}). The chronic toxicity experiments revealed that the toxicity of ALPI increased with increasing exposure time from a 48 h LC_{50} of 18 mg L^{-1} to a 21 day LC_{50} value of 3.2 mg L^{-1}, resulting in an acute-to-chronic ratio of 5.6. This may imply a change in classification from low to moderate toxicity.

DOPO showed only sublethal effects with an EC_{50} value of 48 mg L^{-1} for cumulative reproductive output and an EC50 value of 73 mg L^{-1} for population growth rate. The toxicity of DOPO to *D. magna* was classified as low and likely occurred above environmentally relevant concentrations. The present project obtained a first indication whether or not the selected HFFRs are suitable candidates for BFR replacement in polymers.

426

Prioritization and monitoring of pollutants from emerging thin-film photovoltaic technologies

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Thin-film photovoltaic cells (TF-PV) are a rapidly growing branch among electricity-generating technologies. In particular, price, light-weight structure, semi-transparency and mechanical flexibility offer novel application possibilities. TF-PV can be categorized into well-established inorganic PV (IPV, e.g. copper indium gallium selenide cells, CIGS) and emerging organic PV (OPV). Both represent complex structures composed of manifold organic molecules, metals and metalloids. Despite their potential in renewable energy production, the environmental impact of such TF-PV is still largely unknown. In order to avoid adverse impacts, the most problematic components of TF-PV have to be prioritized, their potential emission monitored, the effects determined and components replaced if necessary. Substances were prioritized as problematic pollutants about which either ecotoxic/bioaccumulation effects are reported (i.e. Cd, Se, Ag) or toxic effects were not yet investigated (i.e. In, fullerenes). Actual emission to environmentally relevant model waters was assessed in laboratory experiments for long-term. In the leachates, metal(loid)s were quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Single organic components of complex leachate mixtures are detected by size exclusion chromatography coupled to organic carbon and light scattering detection as well as high resolution MS techniques (qTOF). From leaching results, predicted environmental concentrations (PEC) were calculated assuming realistic end-of-life leaching scenarios (roof-top acidic rain run-off; dumping in lakes and marine environments). PEC were put into perspective to WHO drinking water limits (human exposure) as well as toxic concentration for aquatic organisms. From the prioritized CIGS and OPV components, in leaching experiments high amounts of Se and Cd were leaching from CIGS. For instance, calculated PEC for Cd (173.4 μg L^{-1}, in acidic rain) would exceed drinking water limits (3 μg L^{-1}) many times and were even acutely toxic for *Daphnia magna* (LC_{50} = 25.5 μg L^{-1}, 48h). In contrast, OPV metal(loid)s did not pose any risk to the environment even under most conservative scenarios. Regarding organic molecules, no fullerenes have so far been detected in the leachates. The lack is probably due to the extreme hydrophobicity (Log K_{ow} = 10.45, determined by HPLC method) of fullerenes wherefore it is not mobilizable in environmentally relevant aqueous leaching tests.

427

Integration of effect-directed analysis in the chemical risk management in a Chemical Industry Park in China

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Chemical Industry Park is a large-scale regional industrial zone mainly for chemical manufacturing, which poses a potential source of risk to human and ecological system. To develop and enhance the technologies of chemical risk assessment and control for the chemical industry parks in China, a series of works has been developed in the Nanjing Chemical Industry Park, as an example of chemical industry park along the Yangtze River. 1) Firstly, a systematic chemical reporting & registration were performed in Nanjing Chemical Industry Park; 2) Secondly, chemicals applied in all manufacturing process were surveyed in over 20 manufacturers in Nanjing Industry Park, and a chemical inventory of 300 chemicals was developed. 3) A database with the physical-chemical parameters, toxicities, and usage data was developed for the chemical inventory. 4) Simultaneously, effected-directed analysis was carried out to investigate the potential hazardous chemicals in the industry park. Water, soil and sediments were sampled from the surrounding area and within the chemical industry park. Ongoing efforts are to identify the chemicals that may cause risk to the environment by the combination of instrumental analysis and bioassays. 5) Finally, tools and methods are being developed to manage the chemicals of high risk based on the information of hazardous chemical storage, production, transportation, and disposal. Here the overall strategies and the progress will be presented and discussed.

428

Finding toxic needles in a haystack: identification of estrogenic chemicals in oil industry process waters using effect-directed analysis

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In addition to the multitude of new synthetic chemicals entering the environment, thousands of unidentified polar compounds derived from the breakdown of oil could also present a hazard to ecosystems. These polar compounds, such as carboxylic acids, traditionally referred to as “naphthenic acids” (NA) within the oil industry, can be present as super-complex mixtures within process waters from oil industries and have been implicated with hormonal disruption. An extreme example of this is the oil sands process-affected waters (OSPW) which result from mining operations in Alberta, Canada. Trillions of litres of OSPW are currently stored in ponds close to the Athabasca river and this is predicted to grow many-fold over the coming years. It is therefore critical that we obtain a better understanding of the nature and toxicity of these waste waters before they enter the environment. Estrogenic activity has been observed in fish following exposure to OSPW but which of the many thousands of unidentified compounds are responsible? Using liquid chromatography (argentation solid phase extraction) followed by tandem gas chromatography – mass spectrometry (GCxGC-MS) together with preparative GC and toxicity tests utilising vitellogenin gene-expression in zebrafish larvae, we were able to significantly limit the number of possible NA structures that could be causing the estrogenic activity. This effect-directed analysis approach has greatly improved our understanding of the nature of NA within OSPW. We now know that although both alicyclic and aromatic acids contribute towards the acute toxicity of OSPW, it is the aromatic acids that are primarily responsible for the estrogenic activity. Further preparative GC will permit greater quantities of individual acids so that improved structural identification can be performed using nuclear magnetic resonance. Within just a few years we are now on the brink of finding the first toxic needles in a haystack that was believed to be impossible to resolve. The Canadian oil sands are just one of many such operations around the globe and NA are present in process waters from oil drilling activities and also result from biodegradation of spilled oil such as from the Deepwater Horizon spill. Pollutants may emerge by being synthetically created but some have always been there but we didn’t realise it due to previous limitations of analytical ability.

429

Identification of emerging pharmaceutical pollutants in a river impacted by an industrial effluent combining passive sampling and effect-directed analysis

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The occurrence of endocrine disrupting compounds was previously detected in a French river impacted by an urban and a pharmaceutical wastewater treatment plants (WWTPs). Indeed, first investigations were conducted in surface water using polar organic compound integrative sampler (POCIS) at upstream and downstream of the WWTPs. Toxicological profiling of POCIS extracts was evaluated using *in vitro* bioassays based on luciferase gene reporter. Strong activities were found in POCIS extracts downstream from pharmaceutical factory. The identification of 60 chemicals (pharmaceuticals, antibiotics and steroids) by targeted chemical analyses (LC-MS/MS) did not totally explain the activities. In order to isolate the active chemicals and to identify them, POCIS crude extract was fractionated using Reverse Phase – High Performance Liquid Chromatography (RP-HPLC). Each fraction was then tested individually on *in vitro* cell lines. Several steroid compounds were detected by target analyses using LC-MS/MS and these chemicals explained the majority of observed activities in the selected fractions. For many highly active fractions, compounds responsible for these activities remained unknown. In this study, a LC-HRMS system (LC-QTOF) was used to identify these active compounds. The HPLC fractions were analyzed in (data-dependent) auto-MS/MS acquisition mode. Detected compounds in fractions of POCIS extract were compared to those found in the corresponding fractions of the blank sample. The characterization of compounds was performed on molecules only present in POCIS fractions. The procedure allowed the identification of several candidate molecules including drugs, steroids and some of their metabolites. The identification of some of them has been confirmed with purchased reference standards. The activity of these identified chemicals is still under process and results will be presented. Acknowledgements: The French Ministry of Ecology, Sustainable Development and Energy, the Aquitaine Region and the European Union (CPER A2E project) are acknowledged for their financial support. Europe is moving in Aquitaine with the European Regional Development Fund.

Biophysical Interactions at the Bio-nano Interface: Relevance for Aquatic Nanotoxicology (II)

430

Colloidal stability dictates the toxicity of nZVI towards the green alga P. subcapitata.

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Aggregation is one among those parameters focusing the attention in Nanotoxicology due to its methodological implications [1, 2]. Aggregation is a physical symptom of a more general physicochemical condition of colloidal particles, that is, colloidal stability. A destabilized colloidal system may tend to reduce its net surface energy by self-aggregation, generating aggregated engineered nanomaterial (ENM) suspensions, but also by hetero-aggregation, including location at bio-interfaces. In this regards, colloidal stability may have an important role as driver of ENM bioactivity. In this study, a spontaneous ENM speciation phenomenon was found when generating a dose gradient of zero-valent iron nanoparticles (nZVI) in algal OCDE culture medium. It consisted of a exceptionally stable ENM suspension with particles in their primary size (4 - 12 nm) which occurred within a tight dose range (0.1-0.5 mg/L). Outside this range, aggregates (150-400 nm) were formed. Interestingly, nZVI exhibited toxicity to the test model organism, except in the 0.1-0.5 mg/L dose range. Stability analyses, TEM images and FTIR analyses revealed that nZVI toxicity was mediated by nZVI-alga interaction, and that the increased colloidal stability of nZVI suspensions in the 0.1-0.5 mg/L dose range was preventing nZVI-algae interaction. Furthermore, destabilization of this particular suspension resulted in toxicity. These observations demonstrate that colloidal stability has a major role in nZVI toxicity, and presumably in ENM toxicity in general. Our results open a new research window on linkages between the stability conditions of ENM suspensions and environmental health and safety (EHS) assessment of ENMs by emphasizing the role of colloidal stability as driver of bioactivity. 1. Handy, R.D., et al., *Ecotoxicity test methods for engineered nanomaterials: Practical experiences and recommendations from the bench*. Environmental Toxicology and Chemistry, 2012. **31**(1): p. 15-31. 2. Schrurs, F. and D. Lison, *Focusing the research efforts*. Nat Nanotechnol, 2012. **7**(9): p. 546-8. *Acknowledgement* - This research was partly supported by Comunidad de Madrid grants S-0505/AMB/0321 and S-2009/AMB/1511 (Microambiente-CM), the Spanish Ministry of Science and Innovation, grant CGL2010-15675 sub-programme BOS, and the US-National Science Foundation, grant number CBET-0853347

431

Platinum nanoparticle toxicity in freshwater algae and crustaceans: A physical or chemical effect?

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Despite intensive research into aquatic toxicity of various nanoparticles, only few studies involve platinum nanoparticles (PtNPs). From an environmental exposure perspective, PtNPs are highly relevant due to extensive use in automobile catalytic converters and the findings of particulate matter containing platinum (Pt) alongside roadways. The aims of this study were to: 1) Determine the toxicity of PtNPs towards freshwater algae and crustaceans, 2) Investigate if observed responses results from physical effects like light obstruction in algal tests and mechanical fixation of organisms in daphnia tests, rather than toxicity induced by PtNPs and 3) Propose alternative test setups to allow for this distinction. The toxicity of $PtCl_4$ and 2 nm starch stabilized PtNPs towards algae and daphnia was assessed. A standard OECD 202 48h acute immobilisation tests with *D. magna* was conducted. In addition, 48h mobility and lethality was assessed in a double-beaker setup with a net separating the daphnia from direct contact with aggregated and sedimented PtNPs. Tests with freshwater green algae *P. subcapitata* included a standard growth inhibition test (ISO 8692:2004) with 48h incubation using fluorometric quantification of algal pigments. Furthermore, a short-term (2h) ^{14}C-assimilation was applied, where algal uptake of ^{14}C-labelled bicarbonate was determined by scintillation counting. Characterization of PtNPs included ICP-OES, DLS and TEM. The tested PtNPs were found less toxic than $PtCl_4$, but still harmful to aquatic life, with 48h EC_{50} values from standard algal and daphnia tests of 14 and 17 mg Pt/L, respectively. The observed 48h algal growth rate inhibition from PtNPs is likely due to a toxic response and not physical shading during pigment analysis, since PtNP toxicity was confirmed in the 2h algal ^{14}C-assimilation test, where scintillation counting is used that is not sensitive to the presence of NPs. However, any shading effects during exposure should be further investigated. In the daphnia test, substantial adhesion of PtNPs to the organisms’ exterior was observed, causing physical rather than toxicity induced immobilization. Consequently, immobilization and lethality endpoints differed greatly, with EC_{50} and LC_{50} values of 17 and 26 mg Pt/L respectively. From the double-beaker setup, comparable EC_{50} and LC_{50} values were 31 and 34 mg Pt/L were obtained, indicating that lethality is a more robust and accurate endpoint than immobility for NPs in *D. magna*.

432

The effect of DOM on the algal toxicity of carbon nanotubes

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With their rapid growth in production and application, carbon nanotubes (CNTs) possess a growing potential for being discharged into the aquatic environment and thereby threaten aquatic organisms via the toxic mechanism which remains unclear. Dissolved organic matter (DOM) is ubiquitous in the environment and may influence the toxicity of the discharged CNTs, which however has not been well investigated. In this study, the individual and combined toxic effects of a type of MWCNTs and three model DOMs (sodium dodecylbenzenesulfonate-SDBS, octyl phenoxy polyethoxyethanol-TX100 and humic acid-HA) to a green alga (*Chlorella Pyrenoidosa*) were investigated by measuring algal biomass, settling curves, malondialdehyde (MDA), reactive oxygen species (ROS), and fluorescence, scanning and transmission electron microscopies (FM, SEM and TEM, respectively). Five principal toxic mechanisms, including oxidative stress, agglomeration and physical interaction, shading effects, metal catalyst residues, and adsorption of nutrient elements, were discussed. Results show that the MWCNTs could significantly inhibit the algal growth under light and dark conditions and the inhibitory effect was dose-dependent. The algal toxicity of the MWCNTs was mainly attributed to the combined effects of oxidative stress, shading effect and physical interaction. The contributions of metal catalyst residues in the MWCNTs and the MWCNT adsorption of nutrient elements to the algal growth inhibition were negligible. Different DOM exhibited different effect on the algal toxicity of the MWCNTs. HA alleviated the algal toxicity of the MWCNTs mainly through reducing oxidative stress and preventing the physical injury of algal cells; whereas the presence of SDBS and TX100 increased the production of intracellular ROS by the MWCNTs, thus enhancing the toxicity.

433

Toxicity of different sized and shaped nanoparticles in zebrafish embryos

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Abstract: Nanoparticles (NPs) are widely used in biomedical, optical and electronic fields. Various studies found Cu, ZnO NPs could increase zebrafish embryos mortality, inhibite hatching, cause changes in viability or morphology, as well as the dissolved ions from NPs. Many studies have provided evidence that particles shape have relationship with the degree of toxicity, although the underlying mechanism remains unclear. Dissolution and shedding of ions also play an important role in NPs induced toxicity. However, there should exist a particle-mediated mechanism. Therefore, we determined whether the particle form of NPs with different shape (ruling out toxicity caused by their shedding ions) have an lethal effect in zebrafish embryos. Three types of Cu NPs with different size, and two types of ZnO NPs with different shape were exposed to zebrafish embryos in egg water. In order to determine whether the particle form of NPs ($NP_{(particle)}$) effect differed from the toxicity of $NP_{(ion)}$, embryos were treated with NPs as well as their nitrate salt solutions. The LC50 values of Cu $NP_{(particle)}$ demonstrated that their toxicity is size-dependent. It can be concluded that smaller Cu NPs were more toxic than larger sized ones, which might be related to the larger surface-to-volume ratio of the smaller particles. Concentrations of shedding Cu^{2+} from Cu NPs were low, and the Cu^{2+} caused less toxicity to zebrafish embryos. Our results showed that Cu $NP_{(particle)}$ dominated the explanation of toxicity. The higher contribution to toxicity by Cu $NP_{(particle)}$ could be caused by the existence of a particle-mediated mechanism. The dissolution characteristics of ZnO nanoparticles play an important role in its toxicity in zebrafish embryos. Ruling out the toxicity caused by Zn^{2+}, The LC50 values of ZnO $NP_{(particle)}$ showed that nano stick was more toxic than nanospheres. The reason could be stick-shaped NPs have a larger contact area with the cell membrane than spherical NPs as the longitudinal axis of the sticks interacts with the cell membrane. Moreover, nanospheres aggregated to large particles, a more effective macrophage clearance for larger particles compared to smaller ones, leading to reduced toxicity of NP aggregates larger than 100 nm. The aggregation of nanoparticles in water is an important factor in assessing their toxicity. **Keywords**: copper and zinc oxide nanoparticles, shedding ions, aggregate state, nanotoxicity

434

Smaller cladoceran species are more vulnerable to CuNPs

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Adverse effects of NPs are largely associated with NP and environmental characteristics. However, little is known about the toxicity of NPs among different NPs and among different species. The prediction of toxicity of NPs to untested NPs and untested species is largely impossible.We propose that interactions between NPs and organisms are target-oriented and which may strongly associated with morphology of organisms [1]. Hence, we exposed five cladoceran species (*Daphnia magna, Daphina pulex, Daphnia galeata, Ceriodaphnia dubia* and *Chydorus sphaericus*) to four sizes of copper nanoparticles (CuNPs, nominal size : 25, 50, 78 and 100nm) to investigate if morphological attributes of cladoceran species can be

used to interpret the toxicity of CuNPs across species. We found that all CuNPs suspensions showed high toxicity to all tested species. Moreover, even though the size, shape and ion release rate of CuNPs were different, the toxicity of CuNPs suspensions and of $Cu(NO_3)_2$ are positively correlated with the body length, surface area and body volume of neonates of different species, indicating the morphological attributes of species could be the parameter which can be used to predict and extrapolate the toxicity of nanoparticle suspension to untested species. Our study shows it is important to include the attributes of species together with the characterization of NPs and offers a new approach to predict the toxicity of nanoparticles for untested species [2]. References [1] Song L, Vijver MG, Peijnenburg WJ, De Snoo GR. 2011. Smart Nanotoxicity Testing for Biodiversity Conservation. Environmental Science& Technology, 45: 6229–6230 [2] Song L, Vijver MG, Peijnenburg WJ, De Snoo GR. In preparation. Acute toxicity of CuNPs suspensions are correlated with the morphological attributes of cladoceran species. *Acknowledgement* - L.S. is sponsored by the Environmental ChemoInformatics (ECO), Marie Curie Initial Training Network within the seventh research framework programme of the European Union (238701). Part of the work was performed within the framework of the RIVM sponsored project “IRAN”.

Bioaccumulation processes and mechanisms: Implications for experimental assessments and modelling (II)

435
Using COSMOmic as a Mechanistic Model to predict Phospholipid-Water Partition Coefficients of Anions

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Membrane lipids are of central importance for bioaccumulation as well as toxicity. The majority of cell membranes consist of phospholipids that form a lipid bilayer – knowledge of the partition behavior of chemicals between water and phospholipid bilayers is thus of special interest. While the partitioning of neutral chemicals can be reliably predicted with empirical $\log K_{ow}$ relationships, the sorption of anions is more complex and not well understood so far. A mechanistic modelling approach is necessary to account for the anisotropy of the membrane. Therefore reliable published data on liposome-water partition coefficients of organic anions have been gathered from literature and were extended with own measurements, using the equilibrium dialysis method. The overall 53 experimental partition data of compounds with a diverse chemical composition shows that there is no constant difference between the sorption of neutral and corresponding anionic forms. An extension of COSMO-RS (Conductor-like Screening Model for Real Solvents) called COSMOmic (COSMO-RS for micelles) has been used to model all experimental data – it is based on quantum chemical calculations and fluid phase thermodynamics. COSMOmic accounts for the anisotropy of the membrane by representing the membrane as a layered structure of consecutive homogeneous liquids. Proven to serve as a mechanistic model for neutral compounds it only needs the atomic distribution of the membrane (via MD simulations) and the structures of every molecule that is involved in the partition system as input files. COSMOmic gives a satisfying statistical correlation when predicting the experimental logarithmic partition coefficients (R2=0.70; RMSD=0.69) with a few outliers. In contrast to the calculations for neutral compounds the COSMOmic-predictions for anions still need to be calibrated with experimental data. It is not fully clear why this additional fitting step is necessary. This fitted prediction for anions is applied to a number of versatile emerging pollutants and the results are compared to a Bioconcentration factor of 2000 and a Biomagnification factor of 1 – with deliberately chosen simple and conservative assumptions. Here it can be shown that at a physiological pH of 7 partially as well as completely ionized organic compounds may have a considerable bioaccumulation potential.

436
Partitioning of POPs into different lipid classes

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Lipids generally represent the major component contributing to the absorptive capacity for hydrophobic persistent pollutants in aquatic ecosystems. Thus, concentrations are often reported normalized to a lipid weight basis in biological samples. The aim of the present study was to determine if contaminants partition in a different degree into two different storage lipid classes, namely wax ester (WE) and triacylglycerol (TAG). This was done by investigating the partitioning of organochlorine compounds (hexachlorobenzene and selected polychlorinated biphenyls) and selected polybrominated diphenyl ethers between lipids (WE or TAG) and a silicone phase. The results suggested that hydrophobic compounds have a higher affinity for WE than for TAG. These results support the hypothesis that contaminants accumulate in a greater extent in Arctic food webs (where WE is an important class of storage lipids at low trophic levels), compared to temperate, and that this can be partly explained by higher water-lipid partitioning at the lower trophic levels. Furthermore, the results suggest that normalizing concentrations of hydrophobic contaminants to lipids in general (e.g. in deductions of trophic magnification factors, TMFs) may be too simplistic, and that information regarding the dominant types of storage lipids in the organisms in question may improve estimates.

437
Abiotic and biotic drivers of Arctic food web contaminant bioaccumulation in a changing climate

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Seasonality in Arctic marine food web contaminant concentrations is known from separate empirical and modeling studies, but the link between the two is missing. The aim of the present study was to identify drivers of seasonal contaminant accumulation pattern in the Arctic marine food web, and to use this knowledge to make predictions of food web accumulation in a changing climate. The effect of the annual cycle of exposure, temperature, lipid content and food web structure on g-HCH, PCB-52 and PCB-153 bioaccumulation in an Arctic marine food web, Svalbard, was modeled using the AQUAWEB model. Results were compared to measured data in the same food web from 2007. The Danish Eulerian Hemisphere Model estimated temperature and contaminant concentrations in air and water in 2007 using real meteorological input data, and projected results for the decade 2090-2099 using climate meteorological data. When varying only contaminant exposure seasonally, delayed peak concentrations moving into the year and up the food web was found, corresponding to empirical data. Modelled bioaccumulation metrics (trophic magnification factor TMF) however, did not correspond with the observed pattern. Best fit scenario between modelled and measured bioaccumulation metrics included highest ecological relevance, allowing also lipids and trophic relations to vary. In the best fit scenario, food web biomagnification varied seasonally, with lowest TMFs in spring, and highest in summer and autumn, as also found in the empirical 2007 study. The results indicate that lipid is not the only important ecological driver of seasonal contaminant accumulation (as previously hypothesised for the Barents Sea), but also food web relations and diet composition (as previously hypothesised for the Arctic). In the future Arctic climate a century ahead, simulated bioaccumulation metrics were marginally higher than at present. Thus, results show that seasonal variation in bioaccumulation is greater than alteration as a response to predicted climate change. As the best fit seasonal scenario included the annual cycle in lipid content and trophic relations, these physiological and ecological factors are crucial for making sound predictions of contaminant food web bioaccumulation for the future. Changes in abiotic drivers alone are not sufficient to explain food web bioaccumulation on a temporal scale.

438
Trophic dilution of cyclic volatile methylsiloxanes (cVMS) across the pelagic food web of a temperate, deep water lake.

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The bioaccumulation behaviours and trophic transfer of cyclic volatile methylsiloxanes (cVMS) and polychlorinated biphenyls (PCB) will be evaluated for the pelagic food web of Lake Champlain, which is a deep water lake located in the USA on the border between the States of Vermont and New York, and extending north into Quebec, Canada. The cVMS included octamethylcyclotetrasiloxane (D4; CAS 556-67-2), decamethylcyclopentasiloxane (D5; CAS 541-02-6) and dodecamethylcyclohexasiloxane (D6; CAS 540-97-6). The PCB included 2,3,4,5,2’,4’,5’-heptachlorobiphenyl (PCB-180) and 2,4,5,2’,4’,5’-hexachlorobiphenyl (PCB-153), which are "legacy" chemicals known to bioaccumulate in aquatic organisms and biomagnify in aquatic food webs. The PCB congeners were used as a benchmark chemical (PCB-180) to calibrate the food web and as a reference chemical (PCB-153) to validate the results. Surface sediments were collected from 60 locations by systematic sampling over a defined study area of 800 km^2 across the main lake basin. Samples of the food web were collected by methods that were deployed to sample the dominate populations of pelagic species. A rigorous quality control program that included reference samples, control samples, and blanks was followed in the field to verify that samples were not contaminated by sample storage and processing procedures. BSAF and TMF will be generated for the sampled food web using $\delta^{15}N$ as a continuous variable for assigning trophic level position. The $\delta^{15}N$ trophic enrichment factor ($\Delta^{15}N$) used to estimate trophic level position will be derived for the food web using PCB-180 as a benchmark having an assumed TMF of 4.0. Standard TMF will be calculated using univariate methods based on linear, least-squares regression analysis. Multivariate probabilistic methods will also be used to calculate TMFs that incorporate natural variability and minimize biases associated with the experimental design, $\Delta^{15}N$, omnivorous feeding, and migration.

The interrelationship between BSAF and TMF will be used to minimize bias and uncertainty associated with TMF and to correct for variable exposure across concentration gradients. Results for Lake Champlain will be compared to results from other studies and quantitative weight of evidence (WoE) methods will be applied to evaluate the impact of experimental design, environment, food web structure, species composition, and exposure on TMF.

439
Limits of transferability of biodynamic parameters to the field for metal bioaccumulation modelling in macro-invertebrates

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The evaluation of the impact of metal contamination on aquatic ecosystems remains important with a biological indices oriented WFD. Determining the exposure to metals by measuring the bioaccumulation in organisms allows assessing more accurately the risk in water bodies. Nevertheless metal bioaccumulation is a complex process dependening on many physiological and physicochemical parameters. The main objective of this work is to investigate the variability of metal bioaccumulation in macro-invertebrates in the field using the biodynamic model (DYMBAM) and a critical appraisal of the published parameters and assumptions. Metal concentrations in Ephemeroptera, Trichoptera, water and Suspended Particulate Matter (SPM) were analysed at 9 sites in Luxembourgish rivers. Both food and animals were also analysed for carbon and nitrogen isotopes. The DYMBAM model was used to make predictions of the internal concentrations in the field species. The C/N isotopic results imply that the same species has a different diet depending on the sites and the environmental conditions. Different diet suggests variable metal contamination and assimilation efficiencies and hence different exposure through food. The DYMBAM predictions using biodynamic parameters from the litterature underestimated the bioaccumulation in the field species. Although the dissolved uptake was the dominant uptake pathway for *Serratella ignita* due to its fast uptake rate from water and low elimination rate, dietary uptake contributed to 80% to the metal bioaccumulation in *Hydropsyche*. These results suggest that dietborne metal uptake is an important pathway for metal accumulation for individual traits like filterers. In addition, the feeding rate and growth of the animals under field conditions is unknown. It would be fruitful to explore relationships between food availability, feeding and growth with energy budget models to reduce these uncertainties. We conclude that metal bioaccumulation in moderately contaminated rivers falls within parameter ranges showing high uncertainties for biodynamic predictions. In light of the importance of dietary exposures in metal accumulation of aquatic macro-invertebrates, metal toxicity depending on dietary uptake routes needs to be investigated more in depth.

440
Terrestrial field studies are needed to derive bioaccumulation potential of chemicals

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The potential of compounds to bioaccumulate in organisms and biomagnify in food webs is key to the regulation of chemicals in several jurisdictions. However, the vast majority of research on bioaccumulation of compounds in food webs (further referred to as “B-assessment”) has been conducted in marine or freshwater ecosystems. This bias overlooks several essential issues with regards to terrestrial ecosystems. In order to asses and optimise the use of data from terrestrial field studies for regulatory B-assessment, a workshop was organised (January 2013) to: i) identify existing knowledge and data on terrestrial B-assessment, ii) provide recommendations to optimise and improve existing approaches by including information from terrestrial studies, and, iii) suggest means of integration of laboratory, model and field derived data. For this, a review was performed on existing data on B-assessment in terrestrial field studies. Additionally, a comparison was made between metrics describing B-potential in aquatic and terrestrial ecosystems. Based on the review, it was concluded that B-bioaccumulation information from aquatic studies are not typically predictive for B-assessment . Participants agreed that this would ignore specific issues of bioaccumulation processes in terrestrial food webs e.g. spatio-temporal variation, bioavailability, physiological-life history differences, etc. Furthermore, bioaccumulation in aquatic food webs is generally driven by the k_{ow} of a chemical; however, in terrestrial food webs persistence of a chemical in various biotransformation outcomes may be more important. It was evident that large data gaps exist and that further research is needed. This research should focus on: i) the use of stable isotopic signals in terrestrial ecosystems, ii) the development of non-destructive/invasive methods, iii) inclusion of bioavailability and metabolism in B-assessment, iv) greater understanding in wildlife physiologically-based pharmacokinetic dynamics, and v) development of modelling approaches to integrate relevant environmental variation in B-assessment. Studies of terrestrial food webs in the field may be challenging, due to ethical but also practical limitations. However, based on the current review it is clear that terrestrial field studies should play an important role in B-assessment.

Mechanistic effect modeling - beyond concentration response and constant environments (II)

441
Mechanistic effect model of a field earthworm and its application to interpret chemical risk assessment field trials

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Although current risk assessments aim to be protective, interactions between chemical, biological and physical stress in nature are generally unaccounted for. Primary decisions on chemical risks to populations are investigated under standardised laboratory conditions. However, field populations are rarely exposed to chemicals at a single dose under constant environmental conditions. At the higher tier field trials can also be highly variable and/or site specific, making interpretation of ecotoxicity results difficult. To addresss these issues we develop and evaluate a mechanistic effect model of the dominant arable earthworm *Aporrectodea caliginosa*. By combining energy budget and agent based modelling (ABM) we describe how populations are altered by both environmental and chemical stress. The energy budget model dictates how individuals assimilate energy from ingested food and expend available energy on maintenance, growth and reproduction. A priori parameter estimates represent individual physiology under specified environmental conditions. Environmental variables in the ABM landscape then act to limit energy expenditure to life cycle processes. Chemicals also alter energy allocation to the physiological processes via imposed dose-response relationships identified from laboratory data. We simulate two chemical risk assessment field trials under both control conditions and following the application of the toxic standard carbendazim. We use toxic standard data here to test whether the model is adequately sensitive to chemical applications. The ABM landscape is set up to represent a vertical cross-section of a soil profile, with environmental factors (food availability, food quality, soil temperature, soil texture and soil water content) varying with depth. We present good model fits to population density and biomass data in both field trials and scenarios simulated. Variability between population dynamics in the field trials is caused by different soil organic matter contents and soil water conditions. Changes in these conditions drive individual movement along the soil profile, essential in capturing the exposure of populations to chemical stress. Combining energy budget and ABMs provide insights into the mechanistic effects of chemicals on field populations living in spatially explicit landscapes.

442
Impact of toxicant stress on the stability characteristics of 2-species competition models

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Classical models of species competition have as outcome competitive exclusion, coexistence or bi-stability (dependence on initial conditions). The same behaviour should be expected in individual-based models (IBMs) including competition. In the Chimera project we coupled IBMs for *Asellus aquaticus* and *Gammarus pulex*, added competition terms to each, and tested whether the dynamics of the resulting model could be approximated with a simpler Lotka-Voltarra competition model (LVM). The particular form of the LVM, as derived from the IBM, was not the *r—K* one with coefficients *r* (per capita growth rate) and *K* (carrying capacity). Instead, the *r-alpha* form resulted with *r* and *alpha* (intra-specific competition coefficient). The LVM was used to analyse the impact of toxicant mortality in single or repeated pulses on the stability characteristics of the system. We found that the IBM showed the same qualitative behaviour as the LVM, in the 4 regions of parameter-space associated with coexistence, exclusion of *Asellus* or *Gammarus*, and bi-stability. Oscillations in the IBM dynamics resulted from non-equilibrium initial conditions (age-structure). In contrast to previous analyses with the *r-K* form, with the *r-alpha* form of the LVM (and thus for the IBM) we found that reduced *r* from chronic exposure (or repeated pulses) could change the stability characteristics. Chronic exposure (or repeated pulses) could convert the competitive exclusion state into competitive coexistence or the bi-stable state into competitive exclusion. A single pulse exposure could affect stability only in the

bi-stable state, if abundance of the winning species was reduced enough to move the system into another basin of attraction. The presence of a competitor slowed down recovery. Details of this 'transient' behaviour depended on context: the state of the sensitive and the competing population, and the stability characteristics of the system as a whole. With two interacting species, toxicant stress acting on one species affects the dynamics of both. Therefore, recovery should take into account return to 'normality' of the whole system, and measures should be based on combined state of both populations. *Acknowledgement* – The Chimera project is financed by the Long Range Initiative of CEFIC (www.cefic-lri.org) (project code: LRI-ECO19)

443

Dynamic modelling and mechanistic effect models to analyse ecological interactions and toxic effects within a microcosm

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In 1998, Clément and coll. worked out a new protocol for a laboratory microcosm bioassay, which has been widely used to assess the toxicity of contaminants. Within the microcosm, toxic effects on classical life history traits are assessed on five species: microalgae (*Pseudokirchneriella subcapitata*), duckweeds (*Lemna minor*), daphnids (*Daphnia magna*), amphipods (*Hyalella azteca*) and chironomids (*Chironomus riparius*). Such an innovative approach allows one to take into account interactions between species and with the abiotic compartment. Within the microcosm, organisms are exposed to contaminants through multiple ways, making difficult to relate observed effects to exposure concentration, which varies in time and space. Moreover, conversely to single-species bioassays where individual responses are directly linked to contaminant concentration, the responses in the microcosm may result from indirect and/or direct effects of the contaminant. Facing such a complexity, modelling appears as a relevant tool to improve results interpretation. Taking into account functional interactions within mechanistic effects models provides a more in-depth understanding of biological responses and a proper way of testing various assumptions on the underlying mechanisms. In this study, experiments were conducted under controlled conditions without and with contaminant (cadmium). The experiments without cadmium were designed to empirically describe the interactions between species. The functioning of the whole microcosm was described through several partial models (for duckweeds/algae, algae/daphnids, etc.) based on ordinary differential equations. Then, effects observed within contaminated microcosms were described through stress functions impacting both species dynamics and interactions between species. Model parameters were estimated using Bayesian inference. Combining each of the partial models without cadmium, we got a complete model with a good description of the global microcosm functioning, with biologically meaningful values for all parameters. The integration of stress functions to describe the effects of cadmium on partial microcosms gave encouraging results to extend the modelling to the whole microcosm with cadmium. Values of interesting parameters without and with cadmium can be compared to quantify the effects within the microcosm, making the microcosm a useful standardized tool for predictive risk assessment.

444

Food web modelling of a river ecosystem for risk assessment of down-the-drain chemicals: a case study with AQUATOX

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Ecological models are a useful platform to assess the impact of chemicals on the structure and function of a whole ecosystem. The fate and effects models AQUATOX is probably the best known example of an integrated fate and effects model combining water quality, food web, chemical fate and ecotoxicological constructs. A case study was developed to simulate the effects of two ingredients of home and personal care products, the anionic surfactant linear alkylbenzenesulphonate and the antimicrobial triclosan, on a lowland riverine ecosystem. The objectives of this study were to construct a realistic food web model of a lowland river ecosystem and to demonstrate how individual-level ecotoxicological data can be scaled up to predict impacts on community and ecosystem dynamics. The model was build for a section of the river Thames (UK), for which detailed ecological surveys were available, allowing for a quantification of energy flows through the whole ecosystem. This offered a unique opportunity for calibration, often impossible for similar models. Key modelling challenges are presented and discussed for each step of the study, including: the definition of a food web structure, the stabilisation of the dynamic food web model and its calibration, the use of the ecotoxicology sub-model and the articulation of a suitable assessment endpoint. The control scenario was calibrated for a simulation period of one year, and tested for stability over six years. Two perturbed scenarios were simulated: one corresponding to environmental realistic concentrations of the chemicals and one with hypothetical inflow concentrations equal to the lowest EC50 value. The difference between the control and the perturbed simulation at environmentally realistic concentrations for the river Thames was statistically insignificant. At hypothetical higher concentrations, direct and indirect effects of chemicals on the ecosystem dynamics emerged from the simulations. Uncertainties in key assumptions remains high as the validation of perturbed simulations remain extremely challenging. The study is a step towards the development of realistic ecological scenarios for use in prospective risk assessment of down-the-drain chemical. The ability to simultaneously simulate the effects of chemicals (including mixtures) and of other water quality stressors associated with domestic wastewater (organic matter, nutrients) enables the evaluation of chemical impacts in realistic, multi-stressed scenarios.

445

Using biological traits in ecological modeling: How to model the temporal dynamics of benthic invertebrate communities?

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Ecological quality of streams has become, with the Water Framework Directive, a central concern in European environmental policy. Ecological quality assessment is only the first step for streams to reach good ecological status. Considering the limited subsidies available, one also have to (i) identify the stressors that could have led to ecological impairment, (ii) predict how the system could respond to new stressors and (iii) predict which management options could potentially lead to the best improvement of ecological quality and recovery of ecosystems function. Mechanistic ecological models that integrate different environmental stressors can provide useful information on the future evolution of systems under changing environmental conditions. The present study details a food-web model (Streambugs) aimed at predicting the seasonal development of the benthic community composition in streams based on temporally variable environmental conditions (e.g. water quality, temperature and floods). However, such models require information for a large set of parameters that is usually scarce. Thus, the identifiability of such parameters from observed data could be a major issue. To limit such problems, one could use a Bayesian approach and include available information on biology and ecology of organisms, the so-called traits, to derive prior information about parameters. We propose to integrate traits in the Streambugs model in two steps. First, as it was previously done, traits related to habitat requirements or to stressor tolerance of taxa are compared to local environmental conditions to derive factors that modify growth and death rates of taxa. As a second step, we propose to analyze if posterior distributions of taxon-specific parameters derived from Bayesian inference correlate with traits that were not yet included in the model. This could help to refine the model to increase its predictive power and universality. With a first manual calibration we got promising results when applying the model to the Sihl river (Switzerland), where we have biweekly abundance data over one year. We will apply Bayesian inference and analyze results as described above to improve the model. Using the calibrated model, we will apply scenario analysis to analyze effects of a pesticide contamination and/or of changing saprobic conditions on the modelled community.

446

Modelling community dynamics under toxicant stress

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In pesticide risk assessment simple and complex experimental setups with aquatic macroinvertebrates are used for risk characterization. However, the combined effects of contamination and biological interactions are often not well enough understood for sound extrapolation to untested conditions. In particular the recovery of populations and communities under realistic conditions is difficult to assess. Here, ecological modelling can support the risk assessment by improving the understanding of the involved processes and feedbacks and by allowing the examination of numerous different experimental settings. Therefore, we developed a dynamic, stochastic, individual-based model (IBM) of a macroinvertebrate community. It is based on the metabolic theory of ecology, that describes the relationship between individual biomass and vital rates. The model incorporates food web interactions including predation and competition (Fig. 1). It describes food uptake, respiration, individual growth, reproduction (including emergence pattern), and mortality. In addition, the model represents individual variability, intrinsic stochasticity and different life-stages of the individuals. For parameter estimation, available trait data bases for macroinvertebrates are used. Here, we present the model and apply it to data of a stream mesocosm experiment to investigating the consequences of toxicant effects on life cycle functions for community dynamics. In particular, we explore the role of community interactions including food competition and predation, going beyond the capacity of single population models.

Policy assessment in an integrated systems perspective: indicators and targets to ensure operating within safe planetary boundaries (II)

447

Environmental assessment of waste management policies: a Waste Input-Output Analysis

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When environmentally assessing waste management policies in a context of economic variations, the interdependence between the flows of goods and waste in the economy needs to be taken into account. Developments in Waste Input Output Analysis (WIOA) are therefore crucial to consistently address such environmental issues. This study aims at i) compiling Waste Input Output Tables (WIOT) for France, combining data in monetary units and data in physical units in a so-called "mixed-unit" or "hybrid" framework and ii) applying WIOA to investigate the environmental benefits of waste management policies on air emissions embodied in the final demand, in the specific case of France and in a context of economic growth. Input Output tables are compiled for France for the year 2004. Coefficients relative to service activities are directly extracted from Eurostat in monetary units, whereas coefficients of products uses, emissions and resource consumption are compiled in physical units mainly from national statistics. Coefficients of waste generation are finally deduced based on mass balance identities. The compiled French WIOT are integrated in a specifically designed web application enabling to perform multiple scenario analyses. Three distinct scenarios are considered, depicting and combining the projected upward trend of final demand from 2008 to 2020, the increase in recycling rates and the larger implementation of Best Available Techniques (BAT) for incineration. The combined implementation of recycling and BAT is observed not to overcome the increase in gaseous emissions induced by the evolution of the final demand. However, their implementation enables to limit the increase in emissions resulting from the evolution of the final demand, by 19% considering fossil CO_2 emissions up to 34% considering SO_2 emissions. Waste management therefore appears an important lever in the perspective of decoupling gaseous emissions embodied in the French final demand from the latter's evolution. Yet waste management policies only have limited effects relatively to the total gaseous emissions embodied in the final demand and may only be seen as one piece of a larger panel of policies.

448

Discussion on methods to include prevention activities in waste management LCA

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Over the last decade, life cycle assessment (LCA) has increasingly been used as a decision support tool in waste management planning at different scale.
Nevertheless, waste prevention has rarely been included in the analysed systems, despite it is indicated as the most favourable option of the so-called waste hierarchy. This may be ascribed to a number of reasons. The first is that traditional waste management LCA has been originally developed with some methodological characteristics that, while simplifying the analysis, prevent its use for the comparison of scenarios where different overall amounts of waste are generated. In this research, the amendments proposed so far in the attempt to overcome this limitation were reviewed first. Two alternative methodological LCA approaches were then identified for the analysis of waste management systems which, beyond conventional treatment methods, include also the effects of waste prevention activities. The two approaches were conceived for the comparison of waste management scenarios including one or more waste prevention activities with baseline scenarios in which no prevention activities are undertaken. For both of them, the functional unit was defined and the system boundaries were described with reference to different typologies of waste prevention activities identified in an extensive review. The procedure for the calculation of the LCA impacts of scenarios was defined as well. The two approaches provide the same result in terms of difference between the LCA impacts of a waste prevention scenario and of a baseline one. However, because of the partially different upstream system boundaries considered for such scenarios by the two approaches, different values of the impacts of single scenarios are obtained. Moreover, their application is more suitable in study with different purposes. The two approaches can be used for many purposes such as, among the most general, quantifying the upstream and downstream environmental consequences of implementing particular waste prevention activities in a given waste treatment system, complementing waste reduction indicators with LCA-based indicators, providing the basis for decoupling evaluations and supporting with quantitative evidence the strategic and policy relevance of waste prevention at national and European level.

449

Taking account of expert stakeholder knowledge: constructing a framework to meet WFD requirements using LCSA and PES for metal removal at abandoned non-coal mine sites.

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The European Water Framework Directive (WFD) requires that water bodies meet good ecological and chemical status. In the UK a significant contributor to surface water pollution, notably Zn, Cd, Cu and Pb, are abandoned non-coalmine sites to which no legal liability for their cleanup is attached. This situation creates a number of different problems which need to be tackled so that individual abandoned noncoal mines can be remediated. These problems include a) identifying sources of funding b) identifying suitable remediation technologies c) justifying the need for remediation to stakeholders and the wider public. A framework has been developed to address these problems and its development has been informed by interviews conducted with expert stakeholders who have connections with or an interest in water bodies or metal pollution. This presentation will present the results of the interviews with expert stakeholders illustrating how different sectors from industry, government agencies and special interest/conservation/stewardship organisations, view this particular issue. Interviewees were asked about their knowledge of and views on payments for ecological services(PES), as a mechanism for funding noncoal mine site remediation, and their opinions on the usefulness of life cycle assessment (LCA) for quantifying the benefits and impacts of the technologies being considered. Their thoughts about what is additionally needed to motivate their organisation to act to cleaner this source of pollution were sought and their knowledge and experience of the current situation was also requested. The similarities and differences of the different sectors perceptions of problems, attitudes and roadblocks will be highlighted in addition to their reflections on potential drivers that would induce their organisation to act to solve this specific problem.

450

Emerging LC Communities around the world: taking the right steps towards sustainable development

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There have been many attemps to make sustainable development operational. Activities around LCA and EPD labeling are coming together as market drivers. The European Commission announced the single market for green products pilot program underway to stsndardize EPDs on consumer goods. Inclusion of LCA and EPDs into the USGBC LEED V4 document is creating a significant demand for LCA services. Several governments are including LCA-based Sustainable Product Purchasing Programs as a step to move the market towards more sustainable practices. This is a welcome development for it opens greater opportunities for environmental and economic development to encompas and transcend this product-by-product approach. ¨Programs like "Life Cycle Communities" created by IERE in 2010 offer much broader action towards sustainability. The goal is to help municipalities use their environmental performance to drive economic growth, redefine policies and sustainable pracitices as well as to use the information developed to obtain EPD labels. An example of the success of this program is the city of Tacoma. Other activities world-wide are following similar approches. Encouraging examples may be found in some leading countries in Latin America. Mexico, Brazil and Colombia are all in the process of implementing Sustainable Consumption and Production (SCP) national policies, including LCAs of priority products in additon to awareness raising, education and progress reporting, amongst other aspects of the SCP programs . In Chile the experience is an EMS certification program for nation wide municipalities, an initiative which to date accounts for at least 10 outstanding communities and which offers the opportunity to gradually move towards LC communities as part of the continual EMS imporvement. ALCALA is currently working in a law project to promote LC communities across Costa Rica as a means to support sustainable development and to contribute to the national goal to be C Nuetral by 2021 in a comprehensive and relaible way. LC Communities have guiding principles: Use LCA and LC Thinking in its policy and operations Have utilities publish their LC Inventories Work with companies to use these inventories for LCA based ecolabels Support educational institutions to build LCA capacity Support SCP practices at all levels Make a public commitment to the above mentioned points (legislation or excecutive order) Continually report to society on performance and improvement

451

Challenges of adding a life cycle perspective to municipal-level decision support for transition to a climate-neutral society

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To address the planetary boundaries for greenhouse gas (GHG) emissions, the EU has adopted the long term target of reducing GHG emissions by 80-95% by 2050. A roadmap has been developed to guide climate policy and wider decision-making towards this target. This work is now spreading to national and local levels. One important part of the road-mapping process is modelling of current and future greenhouse gas emissions in the system under study. The GHG emissions models at municipal level in Sweden has evolved from two parts of the national statistics, (a) the energy statistics at municipal level and (b) the breakdown of national climate statistics to the municipal level. If the aim is to reduce the climate impact of the city, it is not enough to switch from fossil fuels to renewable energy in the local energy mix. There is a need to reduce the climate impact from production of the products consumed by the inhabitants in the city, where life cycle assessment-based data is preferred. The aim of this text is to describe and reflect upon some opportunities and challenges of introducing a life cycle approach to municipal-level to development of future scenarios for long-term, major reductions in greenhouse gas

emissions. The main source of information is an ongoing case study in Uppsala, Sweden. It has been possible to include the LCA perspective for direct energy consumption in the city, but for food and consumer products, there is no agreement on how to include LCA data. Some general concerns from the local stakeholders regarding the use of LCA data in the local GHG accounting and road map are that (a) Figures are uncertain (b) Data are not adjusted to the local context (c) Data are not updated annually. A geographical system boundary, reflecting the boundary of the municipality, risks supporting sub-optimal solutions that transfer of emissions outside the boundary, even though they are caused by activities within the municipality . Production of PV cells is an example from an emerging technology of current local interest. It is not evident that it is possible to merge the geographical and life-cycle perspectives into one model. There may be a need for complimentary modelling activities, which will give a wider picture of the opportunities and challenges of becoming a climate-neutral city. It will however require more resources for modelling and may complicate communication with decision-makers.

452
Development of environmental impacts of Swiss consumption and production from 1996 to 2011
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Human beings consume more and more non-renewable resources, and several renewable resources are being depleted beyond their capacity of regeneration. In a globalised world consumption in one country may cause important environmental im-pacts abroad. This is especially true for small open economies with high shares of im-ported goods such as Switzerland. The extraction, harvesting and processing operations required to produce materials and goods imported into Switzerland contribute to climate change, harm biodiversity and landscapes, pollute soil, water and air in the countries of origin. The study presented here investigates the development of total environmental impacts caused by consumption and production in Switzerland from 1996 to 2011 (Swiss demand based environmental impacts). The study intends to monitor domestic environmental impacts and the importance of foreign trade compared to domestic impacts over time. The development of environmental impacts is compared with the development of population and economic performance. Furthermore, the development of the Swiss impacts due to production and consumption is compared to “planetary boundaries” defined for Switzerland. A few single score impact assessment methods and several impact category indicators covering topics such as climate change, land use, water use and air quality are used to assess the results. The investigation shows that domestic impacts decreased substantially over the last 15 years, mainly due to improvements with regard to air quality. However this reduction is compensated by increasing consumption, i.e. due to increasing foreign trade. Environmental impacts per unit of domestic final demand decrease substantially over the time period analysed. The general trend in Switzerland is towards outsourcing impacts to foreign countries. In developed countries, a responsible environmental policy in view of a green economy should not only focus on domestic environmental impacts but also pay attention to the environ-mental impacts abroad caused by an increasing consumption of goods.

Research on communication and communication of research – pinpointing the best practice to improve our outreach

453
Bridging the Gap: A Case for the use of Social Media in Environmental Science
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In the last few years, social media have gradually become an important part of our daily lives, either through direct contact or through other ‘conventional’ media such as television or radio. While some might see it as a threat or as a source of procrastination, social media have unquestionably changed the way in which information and knowledge disseminate through our society. Science is still struggling to find a way to adopt and integrate social media into its framework. At the same time, more and more researchers are finding their way into mainstream social media such as Facebook or Twitter. We argue that, instead of perceiving this as a problem, scientists should use social media as an opportunity to share and showcase information. This is an opportunity not only to facilitate more informal dialogue about research between scientific peers, but to provide a chance for the general audience to directly interact with researchers. Environmental science in particular could benefit enormously from such an approach, as it deals with topics such as clean air and water, which directly impact our daily lives. By urging scientists to present their research to a wider audience using social media, a more engaging dialogue can arise among all parties. In this context, we organised a professional training course on social media at the Society of Environmental Toxicology and Chemistry North America Annual Meeting in Nashville in November 2013. The purpose of the course was to present environmental scientists with the benefits as well as potential pitfalls of using social media such as Twitter and Facebook in their research. Furthermore, the course provided insights on how to use popular tools such as ResearchGate, LinkedIn, and Mendeley in order to achieve better networking and productivity. In addition to a short overview of our course, we present the feedback received from the course participants on ways in which scientists in our field are using social media, and how they plan to use it in the future. Finally, we present joint social media initiatives among the SETAC Student Advisory Councils from various Geographical Units, and our plans to engage SETAC students in the use of social media for science communication.

454
Blogging: a tool for informal communication of scientific research and technology
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Blogging is a relatively new approach to bridging the gap that exists between scientific research and the public’s understanding of science, that can also serve as a way to enhance scientists’ ability to engage in informal science communication. With the recent advances in science and technology, the need to make newly found knowledge more accessible to the public is ever growing. Today’s digital world offers tools of the Internet that can be effective in communicating and informing the public of new breakthroughs in scientific research and technology. One tool in particular, web logs or “blogs”, have been around since the late 1990s, however, until recently, their emergence as a professional tool for communicating new ideas has been underutilized and underestimated. With millions of people having access to the World Wide Web and an ever increasing disconnect that science and technology has with the general public, blogs offer a cost-effective and user friendly means of reaching a broad demographic of people to increase awareness and educate people of important scientific research and emerging technologies. This presentation will include a discussion of how informal language style effectively communicates scientific research to lay audiences, offering options for starting a blog or blogging, ways to promote blogs and the benefits of blogging for individuals and for collaborative research teams.

455
Uncertainties in environmental nanoparticle research: what is communicated in scientific literature and mass media
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There is a high level of scientific uncertainty in nanoparticle research’ is often stated in the scientific community. The research about the fate and behavior of engineered nanoparticles in the environment is despite its wide applications and years of research still in the early stages. Knowledge about related scientific uncertainties might be of interest to other scientists, experts and laymen. However, a characterization of these uncertainties or knowledge about their scientific communication in scientific literature and mass media are rare. Therefore, the current state of scientific knowledge about scientific uncertainty through the example of environmental nanoparticle research was characterized and the communication of these uncertainties within the scientific literature is compared with its media coverage in the field of nanotechnologies. The scientific uncertainty within the field of environmental fate of nanoparticles is dominated by method uncertainties, a general lack of data and by the uncertain transferability of results to the environmental system. In the scientific literature, scientific uncertainties, their sources, and consequences are mentioned with different foci and to a different extent. As expected, the authors in research papers focus on the certainty of specific results within their specific research question, whereas in review papers, the uncertainties due to a general lack of data are emphasized and the sources and consequences are discussed in a broader environmental context. In the mass media, nanotechnology is often framed as rather certain and positive aspects and benefits are emphasized. Although reporting about a new technology, only in one-third of the reports scientific uncertainties are mentioned. Scientific uncertainties are most often mentioned together with risk and they arise primarily from unknown harmful effects to human health. Environmental issues itself are seldom mentioned. Scientific uncertainties, sources, and consequences have been most widely discussed in the review papers. Research papers and mass media tend to emphasize more the certainty of their scientific results or the benefits of the nanotechnology applications. Neither the broad spectrum nor any specifications of uncertainties have been communicated. This indicates that there has been no effective dialogue over scientific uncertainty with the public so far.

456
Effective environmental risk communication - success stories or urban legends?
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We take part in different risk discourses every day both willingly and in passing. By 2012 the Internet has become the most popular source of risk information[1]. Thanks to social media, we also share and create risk information. But what makes risk communication successful? How do we define success – by the size of the audience, the number of people we manage to convince, the effect of the intervention, or the number of tweets? Despite over 30 years of studies, there is still little agreement among risk researchers about the questions above. For a start, research on risk communication can take three different avenues: the content of communication (risk information), the process (how this information is shared), and the implications of communication (why the information is shared) [2]. Many studies tell the readers what risk communication is and why it is needed, while actual analyses of successful interventions are scarce. Our goal was to establish a set of criteria for risk communication success and find common features of successful risk communication campaigns. We focused on environmental risk interventions, but included different communication formats (e.g. spatial risk maps), as long as there was a clear environmental/ecological risk or threat being communicated. Another important criterion was to include only studies in which a risk communication effect was measured and assessed (e.g., in randomized controlled trials). We excluded qualitative studies, which are difficult to compare. We searched known scientific databases (all to March 2014) in English applying the selection criteria. We also reviewed the reference lists and searched for citations of included studies. Despite the voluminous literature in the area (over 1000 titles), we initially focused on 10 studies meeting our criteria. We identified different aims of risk communication, such as: education, raising awareness, and building trust between communicating parties. So far, the results are not consistent – we were not able to identify one communication format that works better than others in educating people and raising awareness, although simple messages seem to be sufficient for the latter. Risk managers working with probabilities might find it interesting that verbal and graphical presentations of probabilities result in better understanding than numerical ones. In comparison to health and personal risk communication, the area of environmental risk communication still remains understudied. 1. National Science Board: *Science and Engineering Indicators 2012*. Arlington VA: National Science Foundation; 2012. 2. Arvai J, Rivers L: *Effective risk communication*. Taylor & Francis; 2012.

457
Science Communication in the Field of Innovative Human Safety Assessment: The SEURAT-1 Dissemination Strategy
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SEURAT-1 is the major European research initiative in the field of human safety assessment, focussing on the development of animal-free toxicity testing methods. Consequently, a dissemination strategy targetting on different groups (stakeholders, industry, regulators, the public, and policy and opinion makers) were developed, defining specific means for reaching out to these groups. Questions addressed: - How to deal with the conflict of interest between science communication and lobbyism? Is there any? - What are pitfalls for scientists when communicating their results to the public?

458
Pharmaceutical residues in the water cycle: Challenges of communicating an ‘uncertain risk’ to the public
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Pharmaceutical residues in the water cycle are *emerging* anthropogenic contaminants mainly in the sense that there is growing recognition of their potential significance as a risk management challenge. In some countries of the Western World the notion is spreading that a precautionary approach is required in light of studies showing that certain substances can have negative effects in the water flora and fauna and that some substances are even present in drinking water, and given an aging population and an increasing use of several prescription and over-the-counter drugs. Further, the notion is spreading that such an approach needs to include communication to the general public on issues of correct disposal and prudent use of drugs. We have dealt in a literature study with this question: What are content-related requirements of risk communication on pharmaceuticals in the water cycle that aims at sensitization, information and behavior adaptation (drug disposal and use)? So far, there are only few empirical insights into public perception of possible risks of pharmaceutical residues to environmental and human health. Therefore our analysis is based largely on theoretical and empirical insights from the broader literature on risk perception and communication. As a result of the literature study the presentation will point out that the risk source „pharmaceuticals in the water cycle“ has attributes that induce people to perceive a risk as negligible as well as attributes which promote the perception that a risk is high. It is mainly the former attributes that make it likely that part of the addresses have only low motivation to deal more intensively with the offered information. Therefore, *cues* (such as information source credibility) require particular attention in designing the risk communication. Framing the issue broader in terms of „Dealing responsibly with pharmaceuticals“ could serve as an attention cue. The latter attributes, promoting the perception that a risk is high, are main elements of the risk pattern that has been termed „risk as an early indication of insidious danger”. This could be taken account of, for example, by relating emotional cues connected with hazard representations to the aquatic fauna and flora rather than to drinking water. The overall conclusion is that risk communication on pharmaceuticals in the water cycle requires balancing between awakening and reassuring. „It’s worth taking precautionary measures” needs to be the main message.

Environmental OMICs: high-throughput strategies to decipher mechanism of response to stressors (III)

459
Ethynylestradiol (EE2) effect on global gene expression in primary rainbow trout (Oncorhynchus mykiss) hepatocytes
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Implementation of the 3R`s (refinement, reduction and replacement) in ecotoxicology is crucial as to meet the chemical legislations demands of the chemicals that are in need of testing. Through evaluating *in vitro* models potential and implement them as initial chemical screening tools animal testing (*in vivo*) can be reduced. *In vitro* models such as primary cultures and continuous cell lines are well characterized high-throughput screening tools for several single compound and mixtures of endocrine disruptors [1, 2, 4]. Knowledge about endocrine disrupting chemicals (EDCs) different mode of action (MoA) and mechanism of action (MOA) has been obtained through use of batteries of *in vitro* models. Chemicals with ED properties such ethynylestradiol may act as an estrogen mimic by activating the estrogen receptor (ER) through estrogen mediated responses. Activation ofthe ER would initiate a multi-organ endocrine response in vivo, but since *in vitro* lack the full toxicokinetic and toxicodynamic ability of the intact organism, it might have a limitation in its multitude of cellular responses. The aim of this study was therefore to investigate the response in primary rainbow trout (*Oncorhynchus mykiss*) hepatocytes after short-term exposure to the estrogen mimicking chemical ethynylestradiol (EE2) using transcriptomics. Primary hepatocytes exposed to EE2 for 48 hours had a total of significantly 174 differentially expressed gene transcripts (DEG) with a concentration response curve (CRC) between EE2 concentrations, number of DEGs and enriched GOs. The gene ontology (GO) analysis showed that there is an over representation of biological processes related to the nutrient reservoir, lipid related transport and reproduction, potentially associated with production of estrogen related proteins (eg. vitellogenin) in the primary cells. Among all DEGs, 50-67.2% of the rainbow trout genes were successfully mapped to mammalian orthologs. Further, DEGs related to endocrine regulation such as glucocorticoid-, androgen-, Wnt/β-catenin-, calcium-, ERK/MAPK and apoptosis signaling were enriched in these pathways. Primary hepatocytes have shown to be a promising tool in EDC screening as it retains the innate cellular properties of the liver. Using primary hepatocytes and global gene expression as an initial screening tool would contribute to a better understanding of chemical`s MOA in the *in vitro* scenario, and potentially also refining *in vivo* testing of EDCs.

460
Characterization of thyroid metabolism disruption at the level of transcriptome, morphology and behavior
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Accumulating evidence indicates that a large number of environmental chemicals disrupt the normal action of thyroid hormones in human and animals. Disruption of normal TH signaling may have deleterious organismal effects on widespread biological processes. Besides the need for an efficient, low cost, and high throughput routine alternative testing tool, there is also need for further mechanistic information on the effects of thyroid hormone disruption. Using morpholino (MO) technology targeting the different deiodinases (Ds) in zebrafish embryos we provide a reference dataset at the transcriptional, morphological and behavioral level that will be used in an alternative test to identify potential TH disruptors in the environment. Analysis of the impact of D1D2MO and D3MO on a genome-wide scale in head, abdomen and tail at 3 day post fertilization (dpf) using microarrays revealed specific transcriptional patterns. GO analysis revealed that transcripts in the head region of the D1D2MO and D3MO were mainly related to

phototransduction. At the morphological level, impairment of the eye development in knockdown embryos was apparent as soon as 1 dpf, demonstrated by a significantly smaller eye surface. However, this effect persisted up to 4 dpf only in D3 morphants. Further examination of the eye phenotype revealed significantly less cone specific photoreceptors in retinas of D3MO embryos at 3 dpf compared to SCMO. Activity measurement of larvae based on video-tracking at 4 dpf revealed that D1D2 and D3 morphants were significantly less active in light following a dark period, indicating that the knockdown embryos were less sensitive to light stimuli. Besides the observed effects related to eye morphology, other prominent effects were observed in hatching success, swim bladder inflation, heart beat frequency and length. Interestingly, the phenotypes of D3 morphants showed significantly more frequent retardation and defects in early developmental processes compared to D1D2MO embryos. By integrating transcriptional and higher organismal level effects of thyroid hormone disruption we provide important insight on the mechanisms of environmental THDs. Our findings show that visual perception, eye surface, hatching success, swim bladder inflation, length and behavioral profiling are promising endpoints for identification of THDs in the environment.

461

Investigating the hepatic transcriptomic response of brown tout exposed to a model oestrogen, E2, and the anti-androgen, linuron, using RNA-seq

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Brown trout are a sensitive, ecologically important species. 17β-oestradiol (E2) and the anti-androgenic herbicide linuron are two contaminants present in agricultural pollution that are likely to affect brown trout populations. We set out to investigate the global hepatic transcriptomic response of mature male brown trout exposed to E2 and linuron using RNA-seq, which is emerging as a valuable tool for conducting transcriptomics in species with limited genomic sequence information. We exposed mature male brown trout to 3 measured concentrations of E2 (1.94, 18.06 and 34.38 ng/L), 3 concentrations of linuron (2.5, 25 and 250 μg/L) and water controls for 4 days and sequenced three individual liver samples from each treatment on an Illumina HiSeq 2500 platform. Using a *de novo* approach, we assembled a hepatic transcriptome consisting of 172,688 transcripts. Transcript expression analysis revealed a total of 2113 differentially expressed transcripts in fish exposed to 34.38 ng E2/L (measured concentration) compared to the control group, but few changes in the lower treatments. The most up-regulated transcripts included well characterised oestrogen-responsive genes (vitellogenins, zona pellucida proteins, *esr1*), while up-regulated hepatic processes were associated with vitellogenesis (lipid metabolism, cell proliferation, ribosome biogenesis). 34.38 ng E2/L is in a range of values measured in sewage effluent and has been associated with peaks in agricultural pollution, thereby raising concerns for brown trout populations in the most contaminated rivers. 822 transcripts were differentially regulated across all linuron treatments. We identified a striking down-regulation of enzymes involved in cholesterol biosynthesis, possibly resulting from a disruption of androgen signalling. We also found evidence of a considerable induction of transcripts involved in cellular stress response, including up-regulation of CYP1A (up to 560 fold) by 250 μg linuron/L, as well as molecular chaperones and the antioxidant system. There were similar, less pronounced, transcriptional changes in fish exposed to 2.5 μg linuron/L which may raise concerns over the potential effects of this pesticide on wild brown trout populations. These results, which include identification of both conserved and novel toxicological effects in an ecologically important species, also demonstrate the value of RNA-seq as a sensitive and robust tool in ecotoxicology.

462

Developing a platform of environmental omics for the green-lipped mussel Perna viridis

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The green-lipped mussel *Perna viridis* is an important marine biomonitor species in pollution monitoring and ecotoxicological studies in Asia-Pacific region, and considered as a subtropical equivalent biomonitor of the temperate *Mytilus* species. However, the genomic information of *P. viridis* is still largely unexplored when compared with *Mytilus* species. This study aimed to establish a transcriptomic profile of *P. viridis* using the next generation sequencing technology and provide a good representative set of genomic information for elucidation of toxic mechanisms upon pollution stresses and identification of a suite of suitable biomarkers for monitoring marine pollution and environmental stresses. To obtain a wide spectrum of environmental-associated transcripts, adult mussels (4-5 cm shell length) were collected from different locations in Hong Kong and from those after 24-hour exposures to various challenges of physical stresses and chemical pollutants, so as to cover a wide range of stress-associated transcription patterns for future environmental studies. Two males and females from each location and from each treatment were chosen for obtaining the three target tissues (i.e., hepatopancreas, gill and adductor muscle). For each sex and each tissue type, a total RNA sample was extracted from pooled tissues from the field and laboratory treated mussels. The RNA sample was subjected to cDNA library construction, followed by the RNA-sequencing using a Solexa GAIIx (Illumina). Including the splicing variants, a *de novo* assembly of a total length of 295,064,579 base-pair (bp) contig was obtained, with 233,257 contigs assembled of an average size of 1264 bp. The 192,879 non-redundant assembled transcripts were blasted against the NCBI nr database and three molluscan EST databases, and resulted in 44,713 transcripts with at least a blast hit, and having a top match with the sequences from the Pacific oyster, *Crassostrea gigas (*27,651 transcripts). A total of 5,131 transcripts were assigned with KEGG annotation involving in 329 pathways. Based on multivariate statistical analysis, expression patterns of genes from stress associated responses and detoxification were strongly tissue-specific but the differences between genders were little. The anticipated genomic database generated from this study will further strengthen the role of *P. viridis* as a universal marine biomonitor in the Asia-Pacific region.

463

Identification of biomarkers of metal exposure by transcriptome analysis of Elodea nuttallii

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Gene expression analysis by recently developed RNA sequencing technology is a promising tool to be used to develop biomarkers of exposure for use in the field. A good biomarker should be specific and react dose-dependently. Rooted aquatic macrophytes are good candidates for ecotoxicological tests because they are exposed to both water and sediments. *Elodea nuttallii* is such a macrophyte. It is native to Northern America, but occurs almost worldwide as neophyte. It has been shown to accumulate high amounts of toxic metals. In the present study, we used *E. nuttallii* exposed to metals in the lab in order to identify gene signatures that can be used to reveal metal exposure in the field. Using RNA-Seq, we identified more than 60’000 expressed gene sequences, of which about 35’000 could be identified by BLAST searches and 33’000 could be functionally annotated. GO annotation revealed a broad range of biological processes and molecular functions to be present, indicating that we identified a representative gene catalogue of *E. nuttallii*. Expression analysis considering only dose-dependently regulated genes revealed 54 up- and 158 down-regulated by Cd. For Hg we found 84 up- and 86 down-regulated genes. After validating RNA-Seq for a small subset of genes by RT-qPCR, this set of genes was used for designing a codeset for Nanostring nCounter analysis. Shoots were exposed to various concentrations of Hg, Cd, Cu, to the Hg contaminated Babeni reservoir, and to a variety of other abiotic parameters i.e. salinity, cold and darkness, and gene expression signature measured by nCounter. We found two main clusters: samples exposed to high (μg/L range) metal concentration formed one group, irrespective of the metal used for exposure. The second cluster consisted of samples exposed to low (ng/L range) metal concentrations, including samples exposed in the Hg contaminated Babeni reservoir in Romania. Genes identified in the laboratory after exposure to high concentrations were successfully used as biomarkers of Hg exposure in the field in a complex environment. On the other hand, the biomarkers also responded to high concentrations of Cu and Cd in the laboratory, suggesting that more genes would be needed to discriminate between the different metals. In conclusion, we were able to show that a genomic approach is suitable for development of ecotoxicological test and that macrophytes are well suited as test organisms.

464

De novo transcriptomic profile in the gonadal tissues of the intertidal whelk Reishia clavigera

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The intertidal whelk *Reishia clavigera* (formerly named as *Thais clavigera*) is one of the most sensitive species suffering from organotin-associated imposex in their females. However, limited information of mRNA transcriptome has restricted the molecular investigation on such endocrine disruption in this species. By means of Illumina sequencing, we obtained a global *de novo* transcriptome from the gonadal tissues from both male and female of *R. clavigera*, with 197,324 assembled transcripts and 151,684 condensed non-redundant transcripts. A blast hit results from the NCBI’s non-redundant molluscan database showed that 28,948 transcripts were successfully annotated with significant matches at an e-value of ≤ 1e-6. Among them, 1,108 transcripts were assigned to a well-defined gene ontology term.

This first transcriptomic study on the gonadal tissues of *R. clavigera* has generated the fundamental information of mRNA transcriptome that paves the path for mechanistic studies of chemical contaminants (e.g., organotins) on this common biomonitor species.

Soil Biodiversity and Ecotoxicology (III)

465

Revealing the effects of silver-induced selective pressure on soil microbial communities by high throughput sequencing diversity screening

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Silver-induced selective pressure on environmental bacterial communities is likely to become increasingly relevant due to the growing use of silver nanoparticles (Ag-NPs) as antimicrobial agents in biomedical supplies and other commercial products. As Ag from many such applications is ultimately released to the environment, e.g. via the wastewater-biosolids-soil pathway it is important to understand the existing prevalence of silver resistant bacteria and the potential for Ag resistance to spread via the bacterial mobilome. We aimed to explore the effects of Ag on total soil bacterial communities and to identify key changes in relative abundance of bacterial groups after long-term exposure to silver ions. Five Australian soils covering a broad range of physical-chemical properties were exposed to silver for 2 weeks (2W) and 9 months (9M) at concentrations ranging from 50-400 mg/kg of soil while non-treated soils were maintained as controls. Total DNA was extracted from the soils in triplicates and polymerase chain reaction (PCR) based counting (real-time quantitative PCR) and partial sequencing (Illumina MiSeq platform based) of the 16S rRNA gene of Bacteria throughout 100 samples was performed. 16S rRNA gene counts had an immediate significant drop in the 2W samples which either persisted or had recovered in the 9M samples. Sequencing analysis of 1,446,210 sequences revealed an inverse relation of the α-diversity with the 16S rRNA gene counts. Minimal convergence of the community structures was found which could be attributed to the exposure time to silver and the applied dose when samples were collectively analyzed, with the samples grouping according to the soil type of origin throughout statistical tests. However, a major influence of the time of exposure to silver and the applied dose was shown when samples of each of the five soil types were analyzed separately. Distance based redundancy analysis showed that 55-78.1 % of the observed variance could be attributed to these two factors with time of exposure being the more important. Sequences classified into the mycobacterial slow growers were shown to become dominant in the 9M samples of one soil type, irrespectively of the applied dose, according to differential abundance tests. Collectively, our results show that the effects of silver on the total microbial community depend on the pre-exposed communities, and that potentially resistant pathogenic strains can be found in environmental samples.

466

Modification to seedling emergence and growth test to reduce quantities of test materials

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Cost can be a limiting factor in testing potential toxicity of newly-emerging contaminants such as nanomaterials on terrestrial organisms. For example, the effect of potentially hazardous materials on early stage plant growth can be determined using a standard terrestrial seedling emergence and growth test (e.g., OECD Test No. 208, Environment Canada EPS 1/RM/45). In this test, multiple plants (usually 5 or 10 depending on the species) are raised in three or more replicate pots, and growth (shoot and root length and biomass) is recorded 7 or 14 d after planting. Depending on the number of replicates and test material concentrations, the test may require kilograms of soil, and the quantity of the material being tested will be correspondingly large, and possible costly. We present results of a modified terrestrial seedling emergence and growth test in which each plant is grown in a separate receptacle. With this modification, the quantity of soils and test materials required for a terrestrial seedling emergence and growth test may be reduced by more than 60 % compared to the standard growth test. Results are presented for two plant species, two natural soils, and two test materials. These results are compared to those from standard growth tests. The advantages and disadvantages of the modified test are discussed.

467

Non-avoidance behaviour to boric acid could be related with the GABAergic mechanism

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Soil invertebrates, e.g. enchytraeids, are known to to be able to avoid unfavourable conditions, which gives them an important ecological advantage. These organisms possess chemoreceptors responsible for the detection of stressors in the environment, which caninitiate body reactions to stimuli, e.g. avoidance behaviour. Avoidance can be assessed following the standard guideline for earthworms (ISO 17512-1 2008), which can be affected by chemicals. Here we foccused on the non-avoidance of boric acid case study using *Enchytraeus crypticus*, studying a selection of target genes via Quantitative Real Time PCR (qPCR) related to neurotransmission pathways: acetylcolinesterase (AChE) and Gamma-AminoButyric Acid (GABA). Results confirmed that *E. crypticus* was not able to avoid boric acid. Further, as concentration increased there was an increase in the “non-avoidance”, indicating a possible effect at the chemosensorial or transmission level. The affected pathway does not seem to be related with the cholinergic mechanism. Evidences indicated that it could be related with the GABAergic system. The gene measurements pointed to a dose-response to boric acid, which is in agreement with the increase in non-avoidance behaviour.

Keywords: mechanism of response; soil invertebrate, avoidance behaviour, neurotransmissor

468

Expression of metallothionein isoforms in embryos of the terrestrial snail Cantareus aspersus reveals early life stage adaptation to metal stress

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Metallothioneins (MTs) are ubiquitous metal binding proteins with species-specific, stress-protective and metal-regulatory functions. In adult terrestrial snails, two MT isoforms are involved in metal-specific functions like copper (Cu) homeostasis or cadmium (Cd) detoxification (Dallinger et al. 1997), whilst a third, non-specific isoform binds Cd and Cu simultaneously (Hispard et al. 2008). The gene expression of these three MT isoforms, (CuMT, CdMT and Cd/CuMT) has been explored previously in adults of *Cantareus aspersus* (syn. *Helix aspersa*) (Hispard et al. 2008; Höckner et al. 2011). It was found that the CdMT gene was significantly upregulated by Cd, whereas the two other genes did not respond to Cd or Cu exposure at all. Whilst the embryotoxicity of Cd to *C. aspersus* (hatching success after 20 days of exposure) is known (EC50 = 3.5 mg/L; Druart et al. 2012), no data are available on the expression of the three MT isoforms and their contribution to potential protection against Cd in developing snail embryos. The aim of this study was, therefore, first to look at the expression of the three distinct MT isoforms in Early Life Stage (ELS) of *C. aspersus* by detection of isogene-specific cDNAs and secondly, to quantify their Cd dependant gene upregulation by quantitative Real-Time detection PCR. Our results show that the Cd and Cd/CuMT genes, but not the CuMT gene, are expressed in snail embryos from the first day of development. The transcription of the 3 MT isoforms increased with development time, suggesting that the capacities of metal regulation and detoxification may gradually increase throughout the snail embryogenesis. In control embryos, however, the most important isogene seems to be the Cd/CuMT, whose transcription levels exceeded by far the transcript levels of the other two MT genes. This contrasts with the only minor significance of this gene in adult snails and suggests that in embryos, this isoform may play a comparatively more important function in metal physiology. This function seems not to be related to Cd detoxification. Instead, snail embryos responded to Cd exposure by an over-expression of the CdMT gene, whereas the intermediate Cd/CuMT isogene remained unaffected. Overall our data demonstrate the ability of snail embryos to respond very early to Cd exposure by MT synthesis.

469

Cd-sensitive and Cd-tolerant COI haplotypes couples in the standard test species Eisenia andrei (Oligochaeta)

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To test whether metal-resistant and metal-sensitive genotypes could be an inherent part of the genetic make-up of earthworm populations, we used DNA barcoding to identify the three most commonly shared COI haplotypes between 8 populations of the standard test species *Eisenia andrei*. These COI haplotypes (Hap1- Hap3) were subsequently paired up and exposed to Cd in order to assess their differences in sensitivity. A total of six couples were exposed to 0, 25, 50 and 100 mg Cd/kg for 4 weeks at 20°C. The survival, biomass variation and cocoon production of each couple were assessed weekly. Results indicated that couple 6 (Hap3 x Hap3) was

consistently the most sensitive for all endpoints whereas couple 4 (Hap1 x Hap3) was the least sensitive for reproduction. The rest of the couples showed the same responses for all the endpoints assessed. Our findings indicate that earthworm populations may carry intrinsically metal-tolerant or metal-sensitive genotypes. This may explain why a long-term metal exposed earthworm population would show increased tolerance with time. Furthermore, in the context of ecotoxicological testing, our results underline the importance of using genetically diverse population in laboratory testing as earthworm laboratory stocks have been found to be mostly genetically homogeneous. //

470

Ecolotoxicological evaluation of 2,5-furandicarboxylic acid (FDCA), a novel green chemical from Bio-based industry

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2,5-Furandicarboxylic acid (FDCA) is a promising biomass-derived chemical with large market potential as an alternative ingredient for polyethylene terephthalte (PET). FDCA can be synthesized from 5-hydroxymethyl (HMF) derived from fructose or glucose. Chemicals used during this process could be released into environment. So ecotoxicological data are requested to assess the ecological impact. We measured dissipation rates of FDCA and HMF in sterile and non-sterile soil. FDCA and HMF are readily biodegradable in nature soils within days, and are suggested to be less stable than currently used phthalic acid. Subsequently, collembolan *Folsomia candida* were exposed to sterile and non-sterile soil spiked with FDCA and HMF (according ISO guidelines). The results show FDCA and HMF could only generate a significant toxic effect on reproduction at high concentration after removing bioactive microbial community in nature soil by sterilization. In the context of human health, we measured whole-transcriptome responses in the human liver cell line HepG2 exposed to concentrations that elicit 20% cytotoxicity. This analyses shows transcriptional regulation is not affected by FDCA when compared to the control. Comparing to studies on current phthalic acid, our findings indicate FDCA and HMF are biodegradable in natural soil and FDCA rarely affects human liver cells.

Community and ecosystem ecotoxicology (III)

471

Interaction between stress induced by competition and an insecticide on the response of aquatic invertebrates

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In order to study the way ecological interactions influence the effects of chemicals on aquatic invertebrates we performed some experiments using the pairs of *Gammarus pulex* and *Asellus aquaticus,* and *Daphnia* sp. and rotifers as examples. This abstract will only describe the results of the experiments performed with *G. pulex* and *A. aquaticus*, but the poster presentation will address all experiments. Two experiments were performed in small aquatic microcosms. In both experiments 1.5 L glass microcosms were stocked with *G. pulex* and/or *A. aquaticus* which were exposed to 0, 0.15, 0.20, or 0.25 µg/L chlorpyrifos. Experiment 1 lasted for 7 days and included two levels of interaction, one with 10 individuals of *G. pulex,* and one with 5 individuals of *G. pulex* and 5 individuals of *A. aquaticus*. In experiment 2 the effects of intraspecific competition was studied in triplicate microcosms containing *G. pulex* in densities of 5, 10, 15, or 20 individuals, which were exposed to of chlorpyrifos for 21 days. To study the effects of interspecific competition, microcosms containing 5 *G. pulex* individuals and 5, 10 or 15 individuals of *A. aquaticus* were exposed to chlorpyrifos. In experiment 1 the mortality of *G. pulex* was higher in the presence of *A. aquaticus*. This increased mortality resulted in a lower mortality of *A. aquaticus* in the higher treatment. Visual observations confirmed that *G. pulex* and *A. aquaticus* not only compete over the same food source but that they also predate on each other. Surprisingly, both the intraspecific experiment as the interspecific experiment of experiment 2 showed a mediating effect of competition on the toxicity of chlorpyrifos on *G. pulex*. Additional experiments on e.g. the predation of the two species on each other might be performed to explain the interactions observed in both experiments. Also the experiments with *Daphnia sp*. and rotifers will be finalised and evaluated.

Acknowledgement – The Chimera project is financed by the Long Range Initiative of CEFIC (www.cefic-lri.org) (project code: LRI-ECO19)

472

Effects of intra and interspecific competition on the response of aquatic species to carbendazim: the importance of ecological interactions for risk assessment.

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Ecological risk assessment (ERA) of chemicals has the need to increase its ecological relevance to achieve more realistic prediction. In this sense, single standard test organisms lack realism, first because those species may not represent all taxa to be protected; and secondly, ecological interactions are not taken into account. In addition, a fungicide was chosen for the experiments described here since there are fewer studies on theirs impacts in freshwater ecosystem. The present series of studies use two interacting species to explore how important ecological interaction mechanisms as competition influences the responses of the sensitive species to a toxicant. Experiments using small microcosms were performed to assess the ecotoxicological effect of the fungicide Derosal (active ingredient carbendazim) on two interacting populations of: 1) gastropods, *Bithynia tentaculata* and *Radix peregra*; 2) crustaceans, *Gammarus pulex* and *Asellus aquaticus*; and 3) arthropods and non-arthropod zooplankton, *Daphnia magna* and *Brachionus calciflorus*.This abstract describes the results of the first experiment, but the others are in progress and will also be presented orally. The aim of the present work is to understand better how ecological factors modulate direct and indirect population's responses exposed to a toxicant in order to better inform ecological risk assessment. The experiment was design to test the following hypotheses: a) both intra- as interspecific competition will increase species sensitivity and b) intra- and interspecific competition will have a similar impact on this increase of sensitivity. Preliminary results indicate that both, a) the interaction between competition and toxicant, and b) the type of competition have an influence on the response of B. *tentaculata* to carbendazim, although the mechanism is not yet understood. In addition, the immobility shows to have a higher potential as an endpoint to detect effects on *B. tentaculata* than mortality which is most often used. In conclusion, the study shows that more ecological aspects should be included in ERA in order to predict direct and indirect effects at higher ecological scales than the single species one.

473

Interaction between environmental gradients and micropollutants on the structure and functioning of natural phytoplankton communities

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Natural populations and communities in aquatic environments are continuously exposed to unnatural chemicals, discharged in waterways by human activities in amounts and rates that pose serious questions with regards to their impact on the resilience of natural systems. Our goal was to understand how the effects of these chemicals interfere with the processes that maintain biodiversity in natural communities. We used environmentally relevant scenarios, targeted multiple endpoints (physiological responses and community effects), and used natural phytoplankton communities in a field experiment. We sampled phytoplankton from a eutrophic lake and spiked communities with a mixture of 12 chemicals (including phyrmaceuticals and personal care products) in permeable membrane-based mesocosms. Treated and untreated communities were put back in the lake at different depths, over an environmental gradient in physical conditions and availability of limiting resources. Phytoplankton communities were analysed before spiking, and 7 days after the pulse of chemical stress. Experiments were subjected to selection by environmental filtering over the depth gradient, and over time due to changes in water conditions towards a more oligotrophic environment. We studied how exposure to mixtures of water-borne micropollutants interfered with the natural processes of sorting organisms based on their ecological and functional traits. We recorded community response variables, including community-wide distribution of important phytoplankton traits (such as size, fluorescence, coloniality), total biomass (productivity), and functional diversity. Analysis of the factorial experiment revealed that depth was always a significant factor, confirming the importance of this environmental gradient as a selection factor on phytoplankton community structure and functioning. Both depth and dose of micropollutants affected negatively the functional diversity of phytoplankton communities, while the mixture of chemicals induced significant increase in individual and community biomass. Interactions between depth and the mixture of micropollutants occurred frequently and appeared to be negative, indicating an antagonistic interaction between chemical stress and natural environmental gradients on our response variables. Our data suggest that in a multiple stressors context (reality), pollutants may interfere with natural environmental filters and affect biodiversity dynamics in natural systems.

474

The importance of direct toxicity and an altered food quality for fungicide effects on leaf-shredding invertebrates

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Decomposer-detritivore-systems govern the breakdown of leaf litter in streams. In this process, microbial decomposers (especially fungi) are of great importance as they increase the food quality of leaves (i.e. conditioning) for detritivorous macroinvertebrates (i.e. shredders). Fungicides may thus affect shredders by two pathways, namely direct toxicity and an altered leaf-associated microbial community, causing indirect, i.e. food quality-related, effects. To assess the importance of both pathways, the energy processing of the amphipod *Gammarus fossarum* – i.e. feeding, fine particulate organic matter (FPOM) production, and assimilation – was assessed during two 24-d lasting experiments ($n = 65$) using 2x2-factorial designs (factor 1: presence/absence of fungicide(s) in the test medium, factor 2: leaves conditioned in the presence/absence of fungicide(s)). One experiment was conducted with an organic fungicide mixture and one with the inorganic fungicide copper (Cu). Direct exposure to the organic fungicide mixture significantly reduced feeding. In addition, both direct exposure and the consumption of fungicide-exposed leaves significantly reduced the production of FPOM. Most likely this was due to compensational mechanisms to counteract a stress-induced higher energy demand and a lower food quality due to an impoverished microbial community, respectively. In contrast, direct exposure to Cu caused a significantly higher FPOM production, probably to excrete accumulated Cu in the course of detoxification, triggering also a significantly lower assimilation. Moreover, consumption was significantly lower when fed Cu-exposed leaves, which may be a reaction to the higher food quality (i.e. a higher leaf-associated fungal biomass). On the basis of previous studies by the authors, fungicide-induced microbial community changes can be assumed as trigger for the observed indirect effects. Further analyses of the leaf-associated microbial communities are underway to confirm this assumption. Moreover, also the energy reserves of preserved gammarids are analyzed to assess potential effects on animals' physiological fitness. Nonetheless, the presented results already show that besides direct toxicity, indirect, food quality-related effects need to be considered when assessing the risk of fungicides – and other antimicrobial substances – for the heterotrophic food web.

475

Stressor-induced biodiversity gradients: revisiting biodiversity – ecosystem function relationships

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Biodiversity - ecosystem functioning experiments typically inspect functioning in randomly composed communities representing broad gradients of taxonomic richness. We tested if the corresponding evenness gradients and the resulting diversity-functioning relationships reflect those found in ecosystems challenged by anthropogenic stressors. Marine benthic diatom communities were exposed to the herbicide atrazine, and the resulting gradients of evenness and ecosystem functioning (primary production, energy content and sediment stabilization) were analysed. Atrazine exposure resulted in narrower evenness gradients and steeper diversity-functioning relations than random community assembly. The disproportionately large decrease in functioning following atrazine treatment was related to selective atrazine effects on the species that contributed most to the ecosystem functions. Our findings demonstrate how the sensitivity to stress and the contribution to ecosystem functioning at the species level combine in driving biodiversity-ecosystem functioning relationships in ecosystems experiencing anthropogenic stress.

476

Using stable isotope analysis in vegetated flow-through stream mesocosms to study aquatic-terrestrial subsidies

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Emergence of aquatic insects provides a considerable energy subsidy to riparian food webs but may also transport contaminant residues. Therefore riparian food webs are at risk to be adversely affected by aquatic contamination. The objective of the present study was to develop an integrated stream mesocosms test design capable of identifying these inter-habit effects and, furthermore, providing a comprehensive approach for current ecotoxicological testing. We chose the widely distributed web-building spider *Tetragnatha extensa* as a representative species for riparian predators. The present study was performed at 4 of the 16 stream mesocosms at the University Koblenz-Landau, Campus Landau (Germany). Meshed cages were placed above the vegetated stream mesocosms, each comprising a strip of a terrestrial model meadow ecosystem and a part of the respective aquatic stream section. Four individuals of *T. extensa* were placed in each meshed cage for a time period of one month and emerging insects were determined qualitatively and quantitatively in separate emergence traps. The analysis of stable $\delta^{13}C$ and nitrogen $\delta^{15}N$ isotope ratios revealed the trophic relationships of the present stream mesocosm community, comprising emerging terrestrial and aquatic insect species and the predatory spider *T. extensa*. Data analysis of prey and spider samples showed that the overall emergence predominantly consisted of aquatic emergence. Evaluation of the present study indicates that the use of stable isotopes ratios in ecotoxicological stream mesocosm studies can provide a tool to identify contaminant related effects of aquatic pollution on riparian food web structure. Therefore, the inclusion of land-water interactions such as trophic cross-ecosystem linkages in ecotoxicological stream mesocosm studies might be a relevant future application to obtain and create more realistic test scenarios.

Identification and prioritisation of hazardous emerging pollutants (III)

477

Planar-YES in effect directed analysis

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The identification of compounds with advers biological effects in complex environmental samples is one of the main challenges in ecotoxicology and environmental chemistry. The effect directed analysis which combines sample fractionation, biological assays and chemical analysis is one of the main strategies for the identification of bioactive substances. The high performance liquid chromatography (HPLC) is commonly used for the fractionation of complex samples. However, the usage of HPLC for sample fractionation suffers from some limitations. First, after fractionation a high number of samples have to be tested resulting in a limited sample throughput. Second, the mobile phase used for the HPLC might interfere with the biological assay. Therefore, the direct coupling of biological assays with thin layer chromatography (TLC) was proposed as an alternative approach. This direct coupling is already well established for the testing of acute toxicity using bioluminescent bacterial which are applied on the surface of the TLC-plate but not for specific bioassays like the yeast estrogen screen (YES). The objective of the presented study was to develop such a method (planar-YES, p-YES) and to test the hypothesis that the described approach is a valuable and robust screening tool for effect directed analysis. Therefore, the method is challenged with real samples which cover various fields of applications, i.e. analysis of wastewater and sediment as well as the characterization of sunscreens. The advantages of this approach lie in its rapidness compared to an EDA-approach with e.g. HPLC and a subsequent analysis of fractions for biological activity and in its robustness. The method allows for the analysis of effects in demanding matrices. The p-YES supports compound identification by the possibility to quickly falsify alternative compounds and by the direct accessibility of the compounds on the TLC-plate to a subsequent analysis by mass spectrometry. Activity profiles of samples can be generated easily which allow a comparative assessment of alternative processes for e.g. wastewater treatment without a detailed chemical analysis. Taken together, the p-YES-approach seems to have a high potential to be used as a fast and robust screening tool for various applications in effect directed analysis which can be used as a complementary approach to the more common combination of HPLC with a subsequent bioassay.

478

Diagnostic strains of the Salmonella/microsome assay as a potential tool in Effect Directed Analysis – application to a solution-oriented project

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The analysis of previously known mutagenic compounds is not enough to identify all the mutagenic compounds present in environmental samples. Bioassays, like the Salmonella/microsome assay (Ames test), are key elements in an Effect Directed Analysis (EDA) because they can drive the chemical identification of priority pollutants. Specific strains of the Salmonella/microsome assay as a diagnostic tool to help to identify major mutagenic toxicants in the aquatic environment. Some combined groups of strains, such as YG1041/TA98, YG5161/TA1538/TA98 and YG7108/TA1535, provide specific responses to different classes of mutagens. Benzo(a)pyrene, 1-nitropyrene and methylmethanesulphonate were already tested and the the strains showed adequate sensitivity. Water and sediment extracts will be tested in the Salmonella/microsome assay with the microsuspension protocol. The biologically active fractions will be tested in an additional set of higher tier diagnostic to support toxicant identification. The specificities of the different Salmonella tester strains will be exploited to suggest the types or classes of mutagenic chemicals that are present in environmental sample extracts, and chemical analysis will be performed for confirmation of the results. If successful, this approach will be incorporated as a new diagnostic tool in EDA. We intend to identify new mutagenic compounds using the chemical and biological tools proposed, supporting the selection of priority pollutants in an integrated risk based approach. ***Acknowledgement*** – This work is part of the SOLUTIONS Project – FP7-ENV-2013, Grant Agreement 603437

479
Combining MetFrag and PubMed for tackling environmental "Known Unknowns"
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Structure elucidation of molecules based on tandem mass spectrometry is becoming the method of choice in analytical environmental chemistry, e.g. to detect emerging pollutants. Computer assisted interpretation remains a challenging task that still needs improvement to deal with the huge amount of data acquired with more and more upcoming high-throughput methods. In this work MetFrag, a tool used for the structure elucidation of molecules based on tandem mass data, was combined with the number of reference information in the literature. Therefore the scoring of MetFrag was adapted to consider the number of references of PubMed for each candidate and tested on 3521 tandem mass spectra of 377 compounds originating from environmental samples acquired at the Eawag. The results showed that an increasing influence of the number references in PubMed yields an optimum of the median rank of the correct candidates and therewith improved the results of the processed data. PubMed's reference information alone showed to be insufficient to identify the correct compound. However, only the combination of *in silico* fragmentation with MetFrag and the number of references showed the greatest improvement. Additionally, this approach was used to predict the putatively correct compound for Challenge 9 of the CAMSI contest 2013.

480
The SimpleBox Solution Multi-media mass balance model to predict environmental fate and ecotoxic effects of mixtures of chemical substances in EU river catchments
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The EU FP7 project SOLUTIONS sets out to deliver a conceptual framework for the evidence-based development of environmental water policies. This will integrate innovative chemical and effect-based monitoring tools with the realisation of an integrated system of source, emission, exposure, effect and risk models. SOLUTIONS aims to relate proven effects of chemical substances in rivers to known uses of these chemicals, in order to describe and predict (future) effects of emerging chemicals on the ecolgical water quality. We have developed a spreadsheet based mass balance model of river catchments that allows estimating the fate and transport of a wide selection of chemicals and their transformation products. As a test, we have applied our model to chemicals known under REACH to be used in the combined catchments of the rivers Rhine, Meuse and Scheldt.

481
Pollutants of tomorrow and developments in society
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Emerging pollutants are monitored in surface waters since the nineties. With progress in analytical chemistry it is possible to analyse these substances in low concentrations. Which pollutants can be expected if future developments in society are taken into account? Such developments are described in a broad range of scenarios. The scenarios on climate change are well known and published by the IPPC. The UNESCO- WWAP Water Scenario Project refers to future trends in water consumption and water resources. Until now, such implications are discussed only for some aspects. The study presented here is part of the SOLUTION project, a research project supported by the 7th Framework Programme of the EU. Existing scenarios for developments of society are identified and analysed, timelines on the production and consumption of environmentally harmful chemicals are evaluated. The second step assesses whether causal links can be seen between societal development and future pollutants. Potential links as well as trends in pollution and risks are discussed with experts from different disciplines with a broad view to address trends and to develop options for risk management. First results indicate several connections between developments in society and emerging pollutants. Because of demographic change, the population in Europe will have a higher life-expectancy. Concomitantly there will be an increase of pharmaceutical consumption. A second development is the change of climate. The IPPC expects a rise in temperature as well as -the increase of extreme weather events. As a result, the behaviour of chemicals in water will change which will influence the ecosystems. Mobilization of chemicals from sediments might be facilitated by erosion, flood events or rising temperatures. Substitution of problematic substances like phthalates also per- and polyfluorinated hydrocarbons (PFCs) might be a further driver which leads to changes in the pattern of pollutants. **Keywords:** emerging pollutants, scenarios, developments, prediction

Environmental risk assessment of nanomaterials: open issues, pitfalls and recommendations

482
Human health no-effect levels of (nano)particles as a function of their primary size
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As nanomaterials are increasingly used in a variety of consumer products, potentially leading to the releases of engineered nanoparticles, no fully operational tools within the frameworks of risk assessment (RA) or life cycle assessment (LCA) yet exist to allow for comprehensive and robust assessments of their human health risks and impacts. The large number of potentially influential physicochemical properties and the difficulties to define a metric capturing the relationships between these properties and the toxic effects of nanoparticles are important shortcomings. In this study, we developed a methodology that comprehensively draws on the information available in relevant *in vivo* studies to investigate the possible correlations between these properties and the toxicity of nanoparticles. We apply this methodology to inhalation, ingestion and dermal exposures to TiO_2 and nanosilver particles, for which we reviewed 112 and 48 studies, respectively. For each of them, we reviewed nonneoplastic responses to identify the incidence or absence of adverse effects, and we analysed the relationships between these incidences/absences and the particle primary size using multiple linear regressions. Our results show that a statistically-significant, continuous co-variance of the animal no-observed-adverse-effect levels (NOAEL) with the primary particle size seems to exist. These findings can have important consequences for nanotoxicologists and policy-makers as human size-dependent NOAELs can thus be derived and enable refined, case-specific assessments related to occupational and consumer exposure to engineered nanoparticles. In that setting, the application of our results to specific TiO_2 exposure situations resulted in potentially important risks. Yet, these results should be used with caution as large uncertainties exist, e.g. the mismatch between the nanoparticles tested in toxicological studies and the actually released particles. Therefore, our study can be considered an important step forward for achieving consistent assessments of risks and impacts of nanoparticles, but it also highlights important research needs to be addressed before fully reliable impact and risk assessments of nanomaterials can be made.

483
Species Sensitivity Weighted Distribution (SSWD) as screening tool for ecological risk assessment of engineered nanomaterials: The n-TiO2 case study
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Nanotechnology is an emerging field in the area of science and technology. Due to their unique or enhanced physicochemical properties, some engineered nanomaterials (ENMs) such as nanoscale titanium dioxide (n-TiO_2) are suitable for a wide variety of applications in many sectors (e.g. cosmetics, paint). For this reason the market of n-TiO_2 has been exponentially growing to reach 2 400 000 million tons in 2026 only in the US. At the same time, societal concerns regarding the potential environmental and health risks from this material have increased and need to be addressed trough robust risk analysis. Nevertheless, due to significant epistemic uncertainties and methodological limitations the quantitative Ecological Risk Assessment (ERA) of n-TiO_2 is still beyond the state of the art. Therefore we propose a complementary approach for screening-level ERA that provides a quantitative estimation of ecosystem effects from exposure to ENMs. The methodology further develops the Species Sensitivity Weighted Distribution (SSWD) approach by including three weighting criteria (i.e. species relevance, trophic level aboundance and data quality) to address nano-specific needs. This nano-SSWD model was tested with ecotoxicological data for n-TiO_2 including 189 ecotoxicological endpoints (e.g. LCx) for species representative of the freshwater, seawater and soil compartments. The application of the model resulted in estimation of environmental quality criteria (i.e. Hazard Concentration affecting 5% of the species) and ecological risk (i.e. Potentially Affected Fraction of species). These results were then compared to similar results from applying the conventional SSD approach to the same dataset. SSWD were also built for specific trophic levels (e.g. primary producers) and taxonomic groups (e.g. bacteria) thus allowing to identify the more sensitive ones. The SSWD approach resulted to be a valuable tool for a screening-level ERA allowing to incorporate the data quality evaluation and to obtain a cautelative risk estimation (slightly lower than the one obtained with the conventional SSD) although accounting for intraspecies variation (i.e. species

relevance criterion). However, in order to obtain more reliable results, it is suggested to test the approach to a more complete dataset, once available. Finally, further developments envisage the need to combine the weighted SSD approach with a probabilistic one in order to address a higher risk assessment level of ENMs.

484
LCA-RA combined approach by using a Bayesian model: example of the aquatic ecotoxity impact/risk of the Nano TiO2 production.
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Titanium dioxide (TiO_2) has been commercially produced since the early 20th century for its refractive index propriety "Grubb G.F. and Bakshi B.R., 2010". These TiO_2 particles are rutile of 250 nanometers (nm) to 1 micrometer (μm) in diameter. Anatase-phase TiO_2 particles (with a diameter between 1 and 100 nm) are commercialized for several years in the cosmetics industry but also for their photocatalytic properties. TiO_2 nanoparticles offer a big potential to improve the performance of final products but there are unanswered questions about their impacts on human health and the environment. To appreciate the uncertainties regarding the nano TiO_2 potential impacts and risks on the environment we combine Life Cycle Assessment (LCA) and Risk Assessment (RA) approaches. LCA is a chain-oriented tool to evaluate environmental impacts of a product during its entire life cycle, from the extraction of raw materials, via manufacturing and use, to the waste product final disposal. The data concerning the raw materials consumption and pollutants releases to various environmental compartments are identified and quantified in the life cycle inventory analysis step. These data are converted in environmental impact in the Life Cycle Impact Assessment step (LCIA). The purpose of LCIA is to express the impact in term of category indicator which is calculated multiplying the emission with the fate and effect factors of the nanoparticle. Risk is expressed as a product of an exposure probability and the probability of a resulting hazard such as a reduction in survival of a group of organisms or impaired ecosystem function "Kaplan S., Garrick BJ., 1981". LCA and RA both rely on the same type of data, that are on the one hand emissions into the environment and transfer to the different environmental compartments (fate/exposure), and on the other hand ecotoxicological data (effect/hazard). However, high uncertainties remain concerning the fate and the effect of nanoparticles, so a probabilistic approach such as Bayesian network is used in this nano TiO_2 study. The network gives satisfying results and allows direct visualization of the uncertainties. Two main steps of the Bayesian network are presented to study the aquatic ecotoxicological risk of a TiO_2 production. We focus the communication on the structure of the network, consisting of nodes (variables) and arrows (relationships between variables), and on the probability tables of each variable.

485
A proposal for regulation of nano-Ag, nano-ZnO and nano-CuO based on comparison to dissolved metals
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In the recent past a lot has been written about the utmost urgency to elaborate regulations based on the toxicity of engineered nanoparticles. However, developing regulations seems to be a difficult task and an ambiguous topic where errors can occur easily. Writing more reviews going through the same literature again and again will not provide new information but rather create the unfortunate situation of paralysis by analysis, because a review usually identifies new research needs. The task is definitely highly complex, but does this render impossible finding a first pragmatic valuation for regulation purpose? Our study presents a pragmatic way for a potential regulation for three types of metal and metal oxide nanoparticles with respect to their ecotoxicological hazard potential. Nano-Ag, nano-ZnO and nano-CuO are relatively easy soluble particles. We evaluated studies reporting LC/EC50 values for both the nano-form and dissolved metals for the same test system and organism. We thereby establish a database to detect nanoeffects based on total metal concentrations in the test system. For freshwater and terrestrial organisms the vast majority of the ratios is below 1. For freshwater only 9 of 262 data points (3.4%) and for terrestrial systems only 1 of 15 data points (6.7%) have a ratio above one, indicating that the nano-form is in most cases less toxic than the dissolved ion using total metals concentrations as a metric. The maximum ratio observed was 2.4 for freshwater and 1.3 for terrestrial organisms. For nano-ZnO and nano-CuO the distribution of the ratios was similar. For freshwaters the percentage of data points above one was 18.8% for ZnO and 3.3% for CuO. This comprehensive evaluation of the ecotoxicological literature shows that over all published data spanning a wide range of different organisms, different forms of the nanomaterials, test systems and exposure conditions only few combinations of parameters resulted in a toxicity of the nanomaterials that was higher than that of the same mass of dissolved metal. This evaluation shows that OVERALL the toxicity in the vast majority of the cases was les for the nanomaterials than dissolved metals based on total concentrations. Use of the existing regulations for dissolved metals is therefore able to protect in almost all systems also the organisms from nanomaterials. By using a safety factor of two we can protect almost 100% of all organisms in freshwater and terrestrial systems.

486
What are appropriate fate descriptors and modelling approaches to predict environmental concentrations of engineered nanomaterials for risk assessment?
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A comprehensive risk assessment of any substance requires a combination of hazard and exposure assessment. In this context, environmental fate models that predict environmental concentrations play an important role, especially in the case of engineered nanomaterials (ENMs), where measured environmental concentrations are not yet available due to significant analytical challenges. Environmental fate models are well-established tools used for decades in the risk assessment of organic pollutants. They are based on a set of essential chemical property data, namely degradation half-lives in different environmental media and partition coefficients describing a chemical's relative affinity for different environmental phases. However, due to the fundamentally different properties of ENMs compared to organic pollutants, the concepts and models valid for organic pollutants cannot simply be used for ENMs. Here we present a new concept for environmental fate models for ENMs. The environmental fate of ENMs is dominated by aggregation (homo- and heteroaggregation), transformation (e.g. dissolution, surface transformation, degradation of organic coatings) and sedimentation processes. In contrast to organic chemicals whose environmental fate is largely governed by equilibrium partitioning, the fate of ENMs is exclusively kinetically controlled, because ENMs do not reach thermodynamic equilibrium but are present in the environment as unstable suspensions. Here we provide a detailed analysis of the underlying concepts of equilibrium partitioning and colloid science. We discuss several examples from the literature that directly or indirectly prove that partition coefficients cannot be determined for ENMs. An appropriate fate descriptor that can be used however to describe the interaction of ENMs with natural particles in an environmental fate model is the attachment efficiency, α_{hetero}, which describes the surface interaction of ENMs and natural solid particles by quantifying the probability of successful attachment upon collision. In the context of risk assessment it is important to recognize the differences between conventional pollutants and ENMs and to adjust existing methods carefully and adequately to account for the ENM specific properties. The implications of wrongly using partition coefficients in fate predictions for ENM risk assessment can be significant as they misrepresent the fate processes of ENMs and lead to wrong predicted environmental exposure concentrations.

Predicting molecular properties of environmental contaminants

487
QSARINS: a new software for the development and validation of MLR models and QSARINS-Chem: Insubria datasets and new QSAR/QSPR models for environmental pollutants.
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QSAR models, correctly developed and rigorously validated, are highly useful for screening and prioritizing chemicals without experimental data or even before their synthesis in the safe chemical design approach. Their use in regulation is suggested in the European legislation of chemicals REACH,in particular to reduce experimental costs and tests on animals. In recent years particular attention has been devoted to the validation of QSAR models, and the "OECD principles for the validation of QSARs models for their application in regulation" have been established to increase the confidence on the reliability of data predicted by QSAR models. We propose the new software QSARINS (QSAR-INSUBRIA), for the development of Multiple Linear Regression (MLR) models, by Ordinary Least Squares (OLS), and by Genetic Algorithm (GA) for variable selection. This program is mainly focused on the internal and external validation of QSAR models by various statistical parameters. Additional features implemented in QSARINS include tools for explorative analysis of the datasets by Principal Component Analysis, dataset splitting, applicability domain analysis (e.g., detection of outliers and interpolated or extrapolated predictions), consensus modelling, selection of the best model by MultiCriteria Decision Making (MCDM) and various plots. QSARINS is a user-friendly platform for QSAR modeling in agreement with the OECD Principles and for the analysis of the reliability of the predicted data. QSARINS-Chem, a module of QSARINS, includes several datasets of

environmental pollutants characterized by chemical structures (Hyperchem and Mol formats) and several end-points (physico-chemical properties and biological activities). The chemicals with the related data are accessible in different ways (by CAS, SMILES, names, etc.) and their 3D structure can be visualized. Additionally, some QSAR models based on molecular descriptors calculated by the free online software PaDEL-Descriptor are implemented in QSARINS. Among them the Insubria Persistent Bioaccumulative and Toxic (PBT) Index model for the prediction of the cumulative behavior of new chemicals as PBTs. The new PaDEL-Descriptor models can be easily applied for future predictions on chemicals without experimental data, verifying the Applicability Domain to new chemicals without data. The QMRF of all these PaDEL models is available. QSARINS-Chem can be also used as a management tool of custom datasets and models.

488

Predicting Equilibrium Partitioning of organic chemicals – Validation of Existing Models

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As part of the assessment of the environmental behavior of organic chemicals we need models that are able to predict equilibrium partitioning between various phases with only the molecular structure of the solute as input information. A frequently used approach to reach this goal is to predict $K_{air/water}$ (K_{aw}) and $K_{octanol/water}$ (K_{ow}) by increment methods and then use empirical correlations to predict environmental partitioning between water and other condensed phases from the predicted K_{ow}. However, octanol is not an ideal surrogate for many organic phases in the environment. Hence, it is beneficial to use more direct methods such as ABSOLV, SPARC and COSMOthermX that offer a comparably broad applicability. For none of these methods there is much of an independent validation (i.e. with data not used in model calibration) available. Especially a validation with experimental data of high quality for multifunctional complex compounds is rare. Here we present such a validation for up to 270 complex organic chemicals including pesticides, flame retardants, siloxanes and others. The experimental data used was only measured in one research group (our own) and subject to a thorough quality and consistency control. The validation systems include heptane/propylene carbonate, ethylene glycol/1,2-dichloroethane, heptane/2,2,2-trifluoroethanol, and poly(dimethyl siloxane) (PDMS)/water. Though they are not related to environmental partitioning systems, they represent all types of relevant interactions such as hydrogen bonding properties or van der Waals interactions. In our validation we found ABSOLV and COSMOthermX to perform the best with an average root mean squared error (rmse) of 0.80 and 0.79 log units, respectively. Considering the differences of the validation systems, the rmse ranges are relatively small, i.e., 0.64 to 0.95 log units (ABSOLV) and 0.65 to 0.93 log units (COSMOthermX) suggesting a system-independent prediction accuracy. We suggest that these predictive uncertainties can be used as a benchmark for ranking other predictive methods and for defining error margins in decision processes that use results from these methods.

489

Measuring logP>8.5 for risk assessment – a question of standards

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Assessing the (eco)-toxicological effects and fate of chemical substances in the environment requires to know their physico-chemical characteristics like water solubility and logP. From these data some ecotoxicological results can be deduced by application of dedicated QSARs, which are highly desirable to avoid test on animals, reduce study cost and improve throughput of data generation. Our work focuses on evaluating the different approaches allowing to measure or estimate highly hydrophobic logP (greater than 8.5) with a satisfying accuracy. *In silico* models have been investigated, but clearly robust datasets are missing to ensure accurate results. Standard OECD procedures for logP determination encompass logP values from -2 to +8.2 (OECD 107, 117, 123). For higher logP ranges, extended HPLC measurements can be considered. However non-chemically related standards are often used, which leads to significant discrepancy and poor accuracy of the logP value. Considering a properfume (Haloscent®D) with estimated logP > 9.0 as model substance, we have investigated the possibility to extrapolate "Reversed phase-HPLC logK vs. logP" calibration curve to determine its logP and have evaluated the corresponding accuracy. The impact of the standards on the accuracy of the measurement of logP for this very hydrophobic compound has been determined and it has been demonstrated that strict homologues ensure the best accuracy in the logP evaluation. The strong correlation between the retention factor on reversed-phase HPLC and the number of carbon of the homologues demonstrates that extrapolation is feasible at least up to the target compound. From this study the logP of the properfume is 9.7+-0.3. The logP of its homologue with 8 carbons on the alkyl side chain is 7.8±0.3. Extrapolating logP values from the regression between RP-HPLC retention factors vs. logP can lead to accurate results for highly hydrophobic compounds. Using structurally related standards is key to get the best possible accuracy and limit systematic bias that may occur. This approach can be extended to other compounds to generate robust set of data for high logP and ecotoxicological endpoints estimation by QSARs.

490

Influence of pH and cations on the speciation, bioavailability and toxicity of uranium in Lemna minor

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Uranium is naturally present in both aquatic and terrestrial environments. In freshwater its average concentration typically ranges from 0.01 to 6.6 μg/L in various oxidation states and forms. Due to anthropogenic activities its concentration has locally risen to levels that pose potential ecological risks. Uranium toxicity is known to depend on its redox state, speciation and physiological form with the aqueous UO_2^{2+} and UO_2OH^+ hypothesized to be the most toxic species. To assess the impact of different cations or pH on the toxicity of uranium on plants, a growth inhibition test was set up in which *Lemna minor* was exposed to different uranium concentrations ranging from 0.05 μM up to200 μM for 7 days in varying proton or cation concentrations (Ca^{2+}, Mg^{2+}, Na^{2+}, K^+). In general OECD guidelines (226) were followed except for the phosphate concentrations that were kept to a maximum of 0.5 mg/L to limit the formation of precipitating U-phosphate complexes. Toxicity of U expressed on nominal concentrations decreased with increasing Ca concentrations. Changing Mg and K did not seem to influence U toxicity. At the time of writing experiments for different Na concentrations were still on going. Strongest effect was, however, found for the different pH values where the toxicity clearly decreased with increasing pH. The EC50 value increased from 40μM at pH of 5 (the standard pH-level used in the OECD-growth inhibition test) to 110μM at pH 6. At pH 7 and 8 the EC50 could not be established as no sufficient growth inhibition to calculate the EC-values could be obtained within the tested U-concentration range. At pH 6 and 7 U started to precipitate probably as U-phosphate complexes and as such U concentration were lower than expected at these pH values. Further, as expected from literature pH also clearly influenced the speciation of U in the test medium. As modelled by Geochemist WorkbenchÒ, uranyl (UO_2^{2+}) was the most dominant species at pH 5, at pH 7 U-phosphate complexes were mostly present and finally at pH 8 more U-carbonate was formed. The data clearly support the hypothesis that uranyl is the most toxic U-species for Lemna. Toxicity was similar between pH5, and pH6. and at pH6 uranyl levels were lower but higher UO_2OH^+ concentrations were predicted. Hence, the data further indicate that also UO_2OH^+ will contribute to U toxicity in plants. The data obtained in this study will be further used to establish a Biotic Ligand Model for U in plants.

491

Ecotoxicity Analysis of Cholinium-based Ionic Liquids

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Ionic liquids (ILs) are salts with low melting points, liquid at or close to room temperature, and cholinium-based ionic liquids are quaternary ammonium salts with a wide range of potential industrial applications. Based on the fact that the cholinium is a complex B vitamin and widely used as food additive, cholinium-based ionic liquids have been seen as environmentally harmless and thus, accepted as non-toxic, although their ecotoxicological profile is poorly known. The present communication provides new ecotoxicological data for ten cholinium-based salts and ionic liquids, aiming to extend the surprisingly restricted body of knowledge about the ecotoxicity of this particular family. Furthermore, insight was gained in counteracting two widely accepted principles ruling ILs ecotoxicity, the side-chain effect until a cut-off level and the decrease in toxicity with the functionalization of the cation. Given the wide range of structures that can be engineered within the same IL family to respond to function and environmental challenges, assessments allowing the establishment of structure-activity relationships such as that performed here are of great use for the proper design of “optimal” ILs from a technical and environmental point of view, minimizing the array of final ILs undergoing mandatory and costly prospective risk assessment before licensing. The results show that not all the cholinium tested can be considered harmless towards the test organism adopted (the standard marine bacteria *Vibrio fischeri*). Moreover, data suggest that the cholinium family exhibits a different mechanism of toxicity as compared to the imidazolium ionic liquids previously described in the literature.

492

Estimation of toxicological properties using internet accessible models

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QSAR methods became an important alternative to animal testing, to fill data gaps in hazard assessment and in classification and labelling of chemicals (e.g. the U.N. Globally Harmonized System for chemical labelling, or, the European chemical regulation known as REACH). The widely known public available programs are: VEGA, TEST, and OECD QSAR Toolbox. The models have been developed under consideration of OECD principles for validation of (Q)SAR models used for regulatory purposes. In the presentation an accent is placed on VEGA platform and the four toxicological endpoints predicted by CAESAR programs: mutagenicity, carcinogenicity, developmental toxicity and skin sensitization.The predictions for three data sets are discussed: the set of 27 conazoles, the set of Poly Aromatic Hydrocarbon (PAHs), and the set of compounds taken from the CosIng inventory.

Statistical challenges in ecotoxicology

493

Choice and Evaluation of Statistical Models Used to Analyze Ecotoxicity Experiments

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Criteria are presented by which to evaluate statistical models that might be used to analyze ecotoxicity experiments. These include experimental design considerations, control variability, concentration-response shape, goodness of fit, number of parameters to estimate, normalization of responses, and model uncertainty. Distributional properties of model estimates are developed and shown to be important indicators of model quality. In particular, the ability to estimate ECx for values of x ranging from 10 to 50 is explored as a way of demonstrating capabilities and limitations of models to provide regulatory required values is explored. Special attention is given to fish early life stage, fish sexual development, mollusc reproduction, and terrestrial plant experiments. Examples of good and bad modeling strategies from guideline studies are given.

494

Generalized mixed models for ecotoxicology: avoiding pseudoreplication and unnecessary data transformations

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Ecotoxicological data present a number of challenges for analysis: 1) nonlinear responses (e.g., dose-response curves); 2) non-normally distributed data (e.g, count or proportion data); and 3) non-independence of data points. The latter issue, termed ‘pseudoreplication’ in the ecological literature, often arises when there are latent or insidious factors causing some replicates to be more alike one another, such as samples run on the same gel, or fish reared in the same growth chamber. All three of these factors violate key assumptions of traditional linear parametric statistics (ANOVA and regression). Fortunately, recent developments in statistical computing allow the use of generalized linear mixed models (GLMMs): analyses that allow for nonlinear responses with non-normal data (making it a ‘generalized linear model’) and incorporate random effects that account for differential covariance among replicates (thus a ‘mixed’ model containing both random effects and the main fixed effects). GLMMs offer a powerful alternative analytical approach with two major advantages. First, they permit fitting models to the natural distribution of the data (e.g., Poisson distribution for count data, binomial distribution for proportional data), rather than relying on elaborate transformations of dubious effectiveness (e.g., logarithmic, probit, arcsin square root) to achieve linearity and normality. This improves the predictive ability of the model. Second, they allow an explicit accounting of the true covariability structure of the data, ensuring that latent non-independence does not produce erroneous results or improper p-values. We show the advantages of GLMMs using examples from laboratory experiments on endocrine disruption in fishes, illustrating the improvements in inference and highlighting potential pitfalls in designing and fitting GLMMs.

495

MOSAIC_repro: a new user-friendly web interface to analyse bioassay reproduction data directly on-line

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MOSAIC stands for “*MOdeling and StAtistical tools for ecotoxicology*”. In ecotoxicology, bioassays are classically conducted to measure acute or chronic effects of potentially toxic substances on reproduction, growth and/or survival of living animals. MOSAIC is a user-friendly web interface dedicated to the mathematical and statistical modelling of such bioassay data. Its simple use makes MOSAIC a turnkey decision-making tool for ecotoxicologists and regulators. Without wasting time on extensive mathematical and statistical technicalities, users are given advanced and innovative methods for a valuable quantitative environmental risk assessment. MOSAIC is available at http://pbil.univ-lyon1.fr/software/mosaic/. Today, MOSAIC offers two operational tools : (i) MOSAIC_SSD, a tool dedicated to the species sensitivity distribution (SSD) approach aiming at defining safe levels for toxic compounds in an ecosystem through the calculation of the so-called hazardous concentration for *p*% of the species (*HCp*), even when the toxicity values are censored [1, 2]; (ii) MOSAIC_repro, which provides users with a complete statistical analysis of bioassay reproduction data simultaneously accounting for mortality all along the bioassay. For that purpose, concentration-response models are fitted within a Bayesian framework. This presentation will show all features offered by **MOSAIC_repro**, based on several example datasets, all published and chosen among those offered within the web interface itself. Only a minimal statistical background will be given for a better understanding. We will show how to simply enter a dataset by uploading a tabular file containing reproduction data under a simple text format, and also the way results are provided: (i) raw data in two separate plots: one for survival and the other for cumulative reproduction; (ii) the fitted curve superimposed to the reproduction data expressed per individual-day; (iii) parameter estimates with 95% credible intervals (also named Bayesian confidence intervals); (iv) ECx (x= 5, 10, 20, 50) median values with 95% credible intervals. A collection of MOSAIC_repro screenshots will illustrate how MOSAIC_repro is very user-friendly for any user.

496

The development and application of the probabilistic exposure-response space in describing toxicity for ecological risk assessment

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Results from toxicity testing in environmental toxicology commonly are reported as point estimates either from hypothesis testing or regression analysis. In contrast, a risk analysis requires a probabilistic characterization of the exposure-response curve surrounding a target set for decision-making. We propose a derivative of the regression model, the probabilistic exposure response space (PERS), to meet the requirements. The PERS describes the probability distribution associated with a response level, the associated exposure range and the slope of the curve in this region. In PERS the exposure-responsevalue (ER_x) becomes a distribution bound by the upper and lower limits of the response axis, the upper and lower bounds along the concentration axis and the likelihood of each exposure and response combination. As a test of the approach we characterized the PERS for a set of chemicals with varying modes of action and with results of the toxicity tests to daphnia published in peer reviewed journals. Original datasets were kindly supplied by the authors of the papers. A 3-parameter log-logistic model was used to determine the regression and 95 % confidence intervals were generated. For these comparisons the ER_{20} was selected and values corresponding to the concentrations at the low and high range of the value determined. The PERS approach allowed comprehensive understanding of the exposure-response relationships. For example, the concentration of parathion corresponding to an ER_{20} value for immobilization of *Daphnia magna* resulted in effects ranging from 8 to 42 percent. Next the PERS approach was applied to describing the exposure-response relationships between mercury and fish as part of a regional risk assessment. The data from Dillon et al (2010) were analyzed with estimating the ER curve and the confidence intervals. Comparison of these effects levels to the thresholds constructed using NOEC and LOEC values (Depew et al 2012) demonstrated that these thresholds corresponded to generally unacceptable effect levels. The use of PERS allows for an innate integration of probabilistic information into the risk assessment and also places specific criteria for the design of toxicity tests to be used for environmental risk assessment.

497

Hierarchical modelling of species sensitivity distribution: a case study with diatoms exposed to several herbicides

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The Species Sensitivity Distribution (SSD) is a key tool to assess the ecotoxicological threat of contaminant to biodiversity. It predicts safe concentrations for a contaminant in a community. Widely used, this approach suffers from several drawbacks : i)summarizing the sensitivity of each species by a single CEC entails a loss of valuable information about the other parameters characterizing the concentration-effect curves; ii)it does not propagate the uncertainty on the CEC into the SSD; iii)the HCp only indicates the threat to biodiversity (structural response of the community), without any insight about a functional response of the community. We revisited the current SSD approach to account for all the sources of variability and uncertainty into the prediction and to assess the risk from a functional point of view. For this purpose, we built a global hierarchical model including the concentration-response model together with the distribution law for the SSD. We applied this methodology to study the herbicide toxicity on benthic diatoms from lake Geneva. The classical SSD approach is to model the EC50 distribution. Contrastingly, the hierarchical approach aims to model simultaneously the distributions of all the concentration-response curve

parameters. We work within a Bayesian framework which provides the output of the hierarchical model as a posterior joint distribution of parameters for the whole community. From this joint distribution, it is possible to compute an SSD on any x of the ECx without going back to the concentration-response curve. This SSD includes all sources of uncertainty, propagated from the original raw data. Studying the evolution of the HC5 as a function of the x of the ECx, it was found that the confidence interval on the HC5 expands wildly at low values of x. This casts doubts on the usability of the EC10, for instance, to build an SSD to estimate the HC5 setting water quality standards, although the EC10 is one of the endpoints recommended by EU's Water Framework Directive. From model simulations, it is also possible to extract a quantitative indicator of the functional response of the community to the contaminant. Using this indicator, we compared the functional and structural response of a community of diatoms and found that for low concentrations, the herbicide had a stronger adverse effect on the daily growth in fluorescence than on the biodiversity. This new approach offers new insights on SSD.

498

Quantifying uncertainty in trophic magnification factors using Bayesian inference.

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Food web biomagnification is increasingly assessed by estimating trophic magnification factors (TMF) where solvent (often lipid) normalized contaminant concentration is regressed onto the trophic level, and TMFs are represented by the slope of the relationship. In TMF regressions the trophic levels are assumed independent and not associated with variability or uncertainty pertaining to e.g. quantification. In reality, the trophic levels may vary due to measurement error in stable isotopes of nitrogen ($\delta^{15}N$) of each sample, in $\delta^{15}N$ in selected reference baseline trophic level, and in the enrichment factor of $\delta^{15}N$ between two trophic levels (ΔN), which are all needed to calculate trophic levels. While there is a great need in taking these uncertainties into account, there is a separate demand for reducing the uncertainty with the metric itself. In this study we show that by adding knowledge about the structure of the food web we can both estimate all the relevant parameters (diets, enrichment factors etc), thus taking the uncertainty into acount, while also being able to narrow down the variability in the predicted TMF by using Bayesian inference.

Teaching and communicating sustainability – paving the way to a common understanding and meaningful actions

499

Problem Based Learning and sustainability: experiences from teaching LCA at Aalborg University

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Problem-Based Learning (PBL) is a style of active learning based on problem solving. PBL aims at providing university students with flexible knowledge, capacity to self-learning, and skills in problem solving and collaboration. In this context, the present study explores the advantages and challenges that the PBL model offers for developing five key competences in sustainability: (i) system thinking, (ii) interpersonal competence, (iii) anticipatory competence, (iv) strategic competence, (v) normative competences. The study draws on the experiences from PBL activities performed at Aalborg University (AAU), Denmark, and focuses on the teaching of Life Cycle Assessment as a method for sustainability assessment. The objective is providing recommendations for future LCA teaching and learning. PBL activites performed at AAU were evaluated critically to detemine to what extent they addressed and contributed in developing the five competences. Stenghts and areas for improvement were identified. The study is based on direct observation of activities of students and teachers and its scope is limited to the activities on the topic of LCA during the academic years 2006-2013. The study provided examples of how PBL-approaches were used to develop five specific competences in sustainability. It is concluded that -for the case fo LCA teaching at AAU- the PBL model included activities to develop system thinking, interpersonal competence, and normative competence. However, the PBL approach should be strenghtened regarding its application to the development of anticipatory and stragegic competences.

500

Coaching instead of teaching LCA: 20 Years of experience at universities of applied science

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This presentation describes how we teach or better coach LCA. We have been using this approach with success since several years in diverse courses at the level of university of applied science. It involves students in active, projectbased learning, fosters their creativity, and enables their learning in context. The exercise lends itself to developing and exercising all levels in Bloom's Taxonomy of Learning. Over the last 20 years, we have used LCA case studies on real problems with great student involvement and excitement. Our observations have highlighted to us numerous features on the value of case study based coaching. These factors of student learning are: Working with ill-defined problems, handling the unknown, asking the right questions, relevance of context in solving problems, Importance of interdisciplinary team work, responding to diversity in students, values in decision making, practical experience. In summary, the didactic method case study presents various facets of environmental science, engineering, and decision making to the student. It develops systemic thinking, technical content knowledge, interdisciplinary knowledge, decision making skills, and group interaction and communication skills. Pedagogical and motivational factors such as teaching knowledge in context, learning through trial and error, extended periods of observation, seeing the use of the material learned attract and retain all students. The case study setting also lends itself to modification for different levels and stages of students. In general, LCA provides a fertile ground for investigations of this type ranging from semester-long project or design courses to homework assignments in a general education course.

501

Teaching Sustainability in the Packaging & Graphical Industry – Experiences from Teaching in Swiss Professional Courses

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The author is teaching sustainability since more than 10 years in various courses of the Packaging and Graphical industries in Switzerland. Then continuous education – especially by professional courses – has a high significance in the Swiss society; and is important. Switzerland is a country with a high level of apprenticeships and this dual system with a job market oriented apprenticeship is actually a factor of success for Switzerland. One consequence of the dual system – having a large majority passing via the pathway of the apprentice-ship – is that industries need to prepare themselves their future senior staff members. For this, industries established their proper professional in-service courses – like e.g. the packaging industry with the 2-year-course called „Packaging Manager", or the graphical industry with the 3-year-course „Polygraphische Akademie HF TGZ". The objectives concerning sustainability in these courses are similar – i.e. a first part is used to make students aware of the most important aspects of sustainability and the link and relevance in relation to their professional activities; a second step tells the students how to integrate the topic "sustaina-bility" in their daily business activities with respective tools and instruments. It is obvious that within the given timeframe in these courses a comprehensive treatment of the topic "sustainability" however is not possible. In the first part, the objective is to approach students on a very personal and private level – in order to raise awareness for the issue by highlighting „hot" topics like e.g. energy consumption or mobility. The second part is dealing in large parts with the topic of Life Cycle Assessment (LCA) in a way that the students can understand, and "read" such studies – not that they can establish themselves such studies. In this presentation, the author will report from his proper experiences of the more than 10 years of teaching in the two above mentioned courses. He will present similarities and differences between the two industry sectors, encountered during the various courses and highlight the specific requirements, especially in the area of LCA, that this kind of courses is asking for.

502

Using an Experiential Serious Game to Stimulate Life Cycle Thinking in Organisations and in Education

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The United Nations has served as a catalyst to the development of sustainability thinking in education with its promotion of the "Decade of Education for Sustainable Development" between 2005 and 2014. Its concrete application can be seen clearly in academic research highlighting the importance of incorporating sustainability in marketing curricula (Borin & Metcalf, 2010; Bridges & Wilhem, 2008), and the publication of numerous textbooks. The presentation will introduce a new teaching and training method based on the LCA framework. The method is designed to make managers as well as students, and especially in the marketing field, aware of the actual environmental impact of the products/services that they are orwill be dealing with and to show them the positive or negative influence that marketing decisions can have on this environmental impact. The ultimate goal is to make environmental sustainability issues and values integral to marketing decision-making. This novel pedagogical method is based on an educational game called "LCP: Life Cycle Perceptions". LCP was designed as a "serious game" (i.e., a game whose primary purpose is education as opposed to mere enjoyment; e.g., Crookall, 2010). LCP has received the approval of the United Nations Education Program for Development. The presentation will explain the different learning stages of the game. It is an experiential learning game that helps trainees begin to reconstruct the LCA of a product according to their own perceptions and, subsequently, to confront these perceptions with reality. The learning process thus results from illuminating the gap between the LCA reconstructed by the trainees and the actual LCA of the product. Once the trainees have integrated this new scope

of the product life, trainers can, throughout teaching sessions, explain the influence that marketing decisions may exert on the product's environmental impact. The presentation will illustrate why a significant widening of perspective from the traditional marketing view of the product life cycle to the LCA is needed to fully integrate environmental consciousness to marketing and marketing education. It will underline why such a perspective has to be implemented through experiential learning rather than the traditional lecture format.

503

TBD

504

Adapting teaching and communication of Life Cycle Thinking to industrial practitioners and stakeholders

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The broad concept of sustainable development is widespread in the terminology of academia, policy-making, industry and is part of the common vocabulary of the average consumer as well. However, the meaning of "sustainable development" is different depending on the stakeholder and decision-maker, because of a different perception of the goals and underlying actions. It is the role and responsibility of scientific experts and practitioners to ensure that the proper values are associated to the concept, through tailored trainings, tools and communication actions. Historically, sustainable development is closely linked to environmental management, supported by European and national directives. Focusing on environmental management, several approaches can be adopted. Life Cycle Thinking (LCT) has emerged as one of the most appropriate vectors of knowledge and know-how transfer to stakeholders. Regarding industrial stakeholders in particular, our past experience shows that they first need a basic know-how to manage the approaches and to define their environmental strategy, and then they require concepts and tools to support its implementation. Depending on the industrial company profile, more specific requirements arise. Small and medium enterprises (SMEs) tend mainly to focus on a short term return on investment, by deriving some immediate market benefits from the implementation of LCT to their products. For the bigger industrial groups, where often a team dedicated to LCT is available, the requirements are much more diversified and can be more sophisticated as well. For industrial stakeholders working in emerging countries, environmental management is mainly a way to improve their competitiveness on the international market. On the academic side, the requirements are mainly linked to the research and teaching activities. Teachers and researchers require a deep understanding of the LCT methodologies, tools and their final purposes. The aim of this presentation is to review a number of LCT communication and teaching strategies we have been testing with the above mentioned stakeholders' types, to identify and discuss the key elements of success as a function of the target audience and their needs.

Persistent, Bioaccumulative and Toxic (PBT) substances – identification, assessment and regulatory decision making with a special focus on socio-economic aspects

505

State-of-the-art on bioaccumulation and toxicity criteria for non-aquatic organisms within the PBT/vPvB assessment framework

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Current international and European regulatory criteria for PBT assessment of chemical substances are mainly based on toxicity and bioaccumulation data in aquatic species. For example, bioaccumulation assessment is usually addressed by means of quantitative criteria: the BioConcentration Factor (BCF) or BioAccumulation Factor (BAF) in fish and the octanol-water partitioning coefficient (Kow). In some frameworks generic criteria are also recommended such as high (eco)toxicity and detected high levels in biota. However, no cut-off values or guidance is provided for these criteria. The regulatory frameworks and related management actions may therefore potentially fail to identify a number of substances that are bioaccumulative and/or toxic in non-aquatic food chains (exposed through soil and food), but not in aquatic. This issue has been investigated through a review of the legislative status and scientific knowledge on toxicity and bioaccumulation assessment for non-aquatic organisms at international and European level. The review reveals that some persistent, low hydrophobic and poorly metabolised organic chemicals can biomagnify in terrestrial food webs and bioaccumulate in human blood and tissues, although they do not bioaccumulate/biomagnify in aquatic environments. As both Kow and estimated/measured BCF values reflect the equilibrium partitioning of organic compounds between water and biota and do not take into account either the exposure route through the diet and the organism-to-air exchange, these substances may not be classified as bioaccumulative through the current regulatory criteria. Also with regards to toxicity there may be chemicals that are not classified as toxic in aquatic organisms but may be toxic organisms in terrestrial food chains. Although toxicity data from the aquatic system in general is considered to result in a more conservative classification, there are exceptions in the literature where higher toxicity is observed for the terrestrial system. For example, effects of substances with low water solubility may not be detectable through acute aquatic toxicity tests. Hazards may instead be identified through tests with terrestrial organisms exposed through soil or food. Here we present the state-of-the-art in this field including preliminary views on possible incorporation of non-aquatic criteria in the regulatory assessment of PBT substances. The content of this presentation refers to a JRC Scientific and Policy Report (in preparation).

506

PBT classification under EC 1107/2009: Practical issues and a case example of chlorpyrifos

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A number of chemicals, including several organochlorine pesticides, have been identified as persistent organic pollutants (POPs). These chemicals, particularly if they undergo long-range transport (LRT), are identified, and regulated at the global level by the Stockholm Convention. In addition several additional frameworks such as EC regulation No. 1107/2009 assess chemicals for properties that might confer persistence, bioaccumulation, and toxicity (P, B, and T). There is no explicit framework or guidance for classification other than a draft document from SANCO and, unlike other frameworks such as POPs and REACH, EC1107 does not recommend the use of Weight of Evidence (WoE) for assessing data. Using WoE, we have developed a framework to for PBT assessment of current-use pesticides (CUPs) and illustrate this with the organophosphorus insecticide chlorpyrifos (CPY). In assessing P and B for chemicals, the concern is for the general environment, not for a particular local scenario. Because extreme values that are observed in specific situations are not representative of all locations, it is more appropriate to use mean values. Moreover, because many P or B processes are driven by first-order kinetics, the geometric mean value is most appropriate for comparing triggers for classification. Strength and relevance of data were used to select the best data. Specific scoring criteria were developed for each type of exposure, including bioaccumulation in sediment-dwelling organisms, aqueous bioconcentration, dietary bioaccumulation, persistence in soil, sediment, and water. These criteria were based upon OECD methods and scores for individual criteria were summed and the total score for strength used select studies of better quality. Only studies with final scores of ≥50% of the maximum score were used to assess P, B, and T. This presentation will show how this process was undertaken and why we concluded that chlorpyrifos is not a PBT pesticide.

507

Regulation of PBT and vPvB chemicals within REACH: A stock-pollution approach to authorisation and restriction

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The European chemicals legislation REACH aims at ensuring that the risks caused by substances of very high concern (SVHC) will be adequately controlled and that these chemicals "are progressively replaced by suitable alternative substances or technologies where these are economically and technically viable". Authorisation and restriction of SVHCs are key regulatory instruments in order to achieve this goal. An important subgroup of SVHC are persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) chemicals. For decision-making on the authorisation or restriction of SVHCs a socio-economic analysis (SEA) has to be performed, where companies (authorisation) or chemical agencies of member states (restriction) must balance positive against negative impacts of a defined policy scenario and compare the outcomes with alternative policy scenarios. The current conceptualization of the REACH authorisation and restriction process, including the setup of an SEA, ignores that PBT/vPvB chemicals are stock pollutants, causing environmental concentrations to accumulate over time, depending on the emission- and the decay rate of the chemical. As a consequence, pollution damage caused to humans or ecosystems can remain even long after a chemical, or a certain use of a chemical, has been removed from the market. Ignoring stock effects of PBT/vPvB chemicals may therefore result in erroneous and incoherent regulatory decisions on the use/non-use of these chemicals. Our paper suggests a socio-economic model that allows capturing stock effects in an SEA. We show that for PBT/vPvB chemicals the weighing of benefits against costs in an SEA translates into solving an optimal timing problem and we discuss implications for regulatory decision-making on the authorisation or restriction of these substances. By means of a case study (DDT soil pollution) we identify practical steps in order to apply and implement the approach in an SEA.

While our approach does not claim to solve immediately all empirical and practical problems related to estimating and valuing impacts, we believe that it unravels the decision problem underlying to an SEA of PBT/vPvB chemicals. In addition, it offers guidance to socio-economic analysts and decision-makers at the regulatory level on model selection, data compilation and the definition of further research needs.

508

In silico strategies for the screening and prioritization of potential SVHC substances

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Quantitative Structure-Activity Relationships (QSARs) are methods that can be used to estimate the potential toxicity of a compound from its chemical structure. Since they represent a cost effective and fast method to assess a large amount of compound they can play an important role in screening and prioritizing potential harmful compounds. For this reason they can be a valuable instrument to identify potential PBT and vPvB compounds in the framework of the REACH legislation. We will present an integrated strategy based on a series of tools able to handle different levels of details available on the different endpoints. Thses include continuous QSAR models (e.g. for ready biodegradability), fragment based classification models, substance-based tools (by analizing chemical classes distribution with the support of read-across) to property-based tools (by assessing relevant physico-chemical properties related to the property to be modelled). Efforts are focused on the different aspects necessary to identify potential SVHC: persistence, bioaccumulation and toxicity, including endocrine disruptor issue. The final goal of this study is to produce a new flowchart integrating different pieces of information to prioritize chemicals more accurately. A crucial aspect in this integration will be to properly handle the intrinsic uncertainity related to the estiamtions obtained. This means to take into account from one side the fact the not all methods/models available have the same intrinsic reliability. On the other hand, one single model posses a compound specific reliability depending on its applicability domain.

509

Global flow of contaminants: from consumer products in North America to landfills in developing countries

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Despite the increasing concern on the adverse health effects and persistency of halogenated flame retardants (HFRs), the mass of these substances continues to grow in the environment. PentaBDE, OctaBDE and HBCD were added to the Stockholm Convention POPs list in 2009, which resulted in their replacement with other HFRs with similar physical and chemical properties. Here, we investigated the presence of HFRs in ~300 consumer products in the US and Canada. DecaBDE was the most abundant in WEEE and scrap vehicles, while PentaBDE was dominant in foam products. In newer products (< 5 years), DBDPE, TDCPP and Firemaster 550 compounds were the most abundant. Based on the number of in-use PBDE-containing products, the total mass of PBDEs in consumer products estimated to be ~140,000 tonnes in 2013. Although the mass of PBDEs in the use phase declines at the rate of 4-9% per year, it is expected that the total mass of PBDE alternatives in the use phase grows at a faster rate, as more substances in various concentrations are being added to a wide rage of consumer products. Considering that more than 50% of contaminated products are being disposed of in developing countries, more stringent controls on the use and movement of these chemicals are required.

Reduction, Replacement, and Refinement (3Rs): Animal alternative approaches in ecotoxicology and risk assessment (I)

510

Enhancement of fish toxicity QSAR models for predicting acute LC50 and chronic NOEC values for non-polar narcotics: ECHA dossier submissions as an experimental data source

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Under the REACH legislation, acute toxicity test data on fish are required for chemicals imported into or manufactured within the EU in quantities greater than 10 tonnes per year. For chemicals imported or manufactured in quantities greater than 100 tonnes per year, chronic fish toxicity data are also required. The high number of tests required across the chemicals industry raises costs to both industry and consumers alike in addition to raising animal testing and welfare concerns. The REACH regulations encourage the use of non-testing methods including QSAR. ECOSAR™ is one of the most widely used models to predict aquatic toxicity to fish, daphnia and algae. The methods used to develop the models within ECOSAR™ do not currently meet best practice guidelines for QSAR development and shortcomings have been identified within the scientific literature. In light of this, updated models were developed according to best practice guidance in QSAR creation. Data were taken from multiple sources including the ECHA dissemination portal. As of November 2013, the ECHA dissemination portal contains over 10000 unique substances, with over 41000 dossiers. It has therefore become a very large experimental data source. The reliability and use of this data in model development was evaluated. The models developed meet the OECD principles, have strong internal and external validation statistics, and can provide reliable predictions of the acute/chronic toxicity of non-polar narcotic chemicals towards fish. Whilst some issues with dossier misinformation were discovered, it was found that overall the ECHA dissemination portal is a valuable and reliable data source. When queried using the eChem portal, chemical dossiers containing suitably reliable data, relevant to the endpoint of interest, could be quickly found. The ECHA dissemination portal holds great potential for future QSAR development and improvement if used appropriately.

511

Development of a high Accuracy model for determining the aquatic toxicity of mixtures

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Abstract To date the only recognized method available for measuring mixture toxicity is the Water Accommodated Fraction (WAF) test. In this study, organisms are exposed to a specific mixture concentration termed a “loading”. The quantity of each constituent in water can be measured at a given concentration (loading rate) but this does not always correspond to the original mixture concentration and so it is difficult to demonstrate experimentally that the technical obligations required to maintain the stability of the test substance have been met. For this reason it is difficult to replace experiments accurate model predictions. The determination is complexified by the fact that liquids may partition into each other thereby reducing bioavailability of all the constituents in the aqueous mixture. In order to create a functional WAF model, these aspects therefore needed to be accounted for. The approach used was to create a three step model: First a thermodynamic mixture algorithm was prepared which determines the actual concentration of each constituent in the aqueous phase from a given original mixture concentration; Next, the non-bioavailable phase was accounted for and excluded from the aqueous concentration; Finally, the new algorithms from the ecotoxicity QSAR were used as above to determine the relative toxicity of each constituent in terms of activity. The WAF model was validated using a series of WAF tests on fish. The results were found to be in high agreement with the experimental data.

512

A 3R compliant testing strategy to predict chronic fish toxicity

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To ensure an ecologically relevant basis for environmental quality standards, whole-organism vertebrate tests are considered most suitable. Currently, the Fish Early Life-Stage (FELS) test (OECD 210) is used to estimate chronic toxicity of regulated chemicals to fish. However, the FELS test uses high numbers of animals, is a low throughput test and lacks mechanistic information. We present a workflow combining several 3R compliant testing methods such as *in vivo* alternative and *in silico* methods to develop a mechanistically based alternative testing strategy for prediction of fish chronic toxicity based on the adverse outcome pathway (AOP) framework. We put forward the 5 day ZFET (ZebraFish Embryo Toxicity) test as a valuable alternative *in vivo* test system. We consider three key elements of this alternative testig strategy. Depending on the AOP, QSARs may offer a first source of information. We performed a meta-analysis correlating acute and chronic fish toxicity data available from public databases to acute and chronic ECOSAR toxicity predictions for a set of non-polar narcotics. We observed stronger correlations between experimental and predicted toxicity data for early life-stages compared to other life-stages. We propose to use the 5 day ZFET test to confirm agreement between acute experimental toxicity and QSAR predictions for specific chemicals in order to decide whether the chronic QSARs may be applicable. Secondly, if there is a consistent relationship between acute and chronic early life-stage toxicity for a specific AOP, acute toxicity data gathered using the 5 day ZFET test may be used as a starting point for the prediction of FELS toxicity. We

constructed correlations between acute and chronic fish toxicity data of non-polar narcotics. Again, the correlations were stronger when the data was limited to early life-stages. Next to the already existing concept of acute-to-chronic ratios (ACR), we propose the acute-to-chronic surface (ACS). The ACS forms a rectangle ranging between the minimal and maximal available values of acute and chronic fish toxicity and therefore informs on the uncertainty of the ACR. Finally, to substantially increase the biological relevance of risk assessment, we propose to add a refined 5 day ZFET test including AOP-specific endpoints. We will investigate the predictive potential of endpoints in the 5 day ZFET test representing key events along AOPs.

513

Can adverse outcome pathways help to predict early life-stage toxicity in zebrafish?

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The fish embryo acute toxicity test (FET) has been proposed as an alternative to acute fish toxicity testing (OECD 203) and a test guideline (OECD 236) has recently been approved. Hence, the FET now provides the opportunity to be applied in prospective chemical hazard assessment and whole effluent monitoring for acute toxicity. However, embryonic development of fish is also an integral part of the fish early life stage (FELS) test (OECD 210), which is used for chronic toxicity assessment. Thus, it can be asked whether the information gained in early developmental stages could be used to predict effects in the FELS. To test the suitability of the adverse outcome pathway hypothesis to support such a scope, a collation of well-defined substance data including both acute fish toxicity and FELS data was performed. Ideally, acute to chronic toxicity ratios (ACR) and data for cumulative toxicity (increasing toxicity over time) would be retrieved from a single FELS test. Unfortunately, toxicity in the embryonic period during an FELS is often not easily available from publications or public databases. Therefore, we have initiated setting up a database of time-resolved FELS effect concentrations using published data and registration dossiers. At present, about 100 compounds could be retrieved with data for fish acute toxicity and FELS toxicity for the same species and fulfilling a set of quality criteria. Most data show a clear correlation of FELS and acute fish toxicity data with a systematic sensitivity shift of less than one order of magnitude towards FELS data. Outliers from this correlation (i.e. compounds with a high ratio of acute fish to fish early life stage toxicity) were identified and included, for instance, compounds with a neurotoxic mode of action. This mode of action could also be identified from fish embryo tests using behavioural analysis. Given the principally high correlation of fish embryo and adult fish acute toxicity, we suggest neurotoxicity and / or changes in embryonic behaviour as one potential indicator and predictive endpoint for enhanced chronic toxicity in the FELS to be tailored in further investigations.

514

Inter-laboratory validation of the Xenopus Embryonic Thyroid Signalling Assay

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With support from France and financial participation from Watchfrog and the French ministry responsible for ecology, a validation plan has being developed for the Xenopus Embryonic Thyroid signalling Assay (XETA); laboratories from OECD member participate in the validation study, including laboratories from Europe, North America, and Asia. The objectives of the validation are : 1) to establish the relevance of the assay by assessing its sensitivity to detect disruption by compounds active at different points within the thyroid system, and 2) to assess the reproducibility of the assay across participating laboratories. XETA is an aqueous assay based on the genetic detection of a chemical’s impact on transgenic *Xenopus laevis* embryonic stages. This transgenic line can detect the activity of Thyroid Hormone (TH) agonists that activate TH receptors, as well as antagonists of thyroid axis that work through various mechanisms. The XETA provides a rapid response (The ring test experiments within the three participating laboratories will be completed early 2014. The lead laboratory (WatchFrog) will then collect the raw results and perform the overall statistical analyses. Reliability, reproducibility within and across labs, and sensitivity of the assay will be determined. The minimal number of tadpoles that can be utilised in the test will be determine through statistical evaluation. In addition to serving as a quick screen for thyroid active chemicals, XETA, could serve as a potential alternative method to the *in vivo* Amphibian Metamorphosis Assay (AMA - OECD guideline n°231).This test is based on the study of *X.laevis* tadpole metamorphosis after 3 weeks of exposure to a given chemical, and includes histology of the thyroid. The OECD TG 231 is the only test currently available to assess the thyroid disruption on a whole vertebrate organism. XETA could provide an alternative test that can be performed quickly, providing information that would be useful for screening. It is intended that this assay will be applied to testing in the context of REACH and other international testing programs.

What do we know about the effects of multiple stressors and community responses on aquatic ecosystems?

515

GLOBAQUA: Managing the effects of multiple stressors on aquatic ecosystems under water scarcity

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Water is the most essential of all natural resources and consequently water and its services are major components of the human wellbeing and major factors of socio-economic development in Europe. Nowadays, freshwater ecosystems are under threat due to a great variety of stressors that can have deleterious effects on them, including pollution, geomorphological alterations, land cover change, invasive species and water scarcity. In order to properly address the effect of stressors in policy terms, a coordinated effort on the research that considers multiple perspectives is needed.Within this context GLOBAQUA project has assembled a multidisciplinary team of leading scientist in the fields of hydrology, chemistry, ecology, ecotoxicology, economy, sociology, engineering and modelling in order to study the interaction of multiple stressors within the frame of strong pressure on water resources. As one of the last FP7 projects, GLOABAQUA, with the full title “Managing the effects of multiple stressors on aquatic ecosystems under water scarcity” is a 5-year project that started on February 2014. The consortium is composed of 21 European partners from 8 countries and 2 non-EU partners from Morocco and Canada. The team includes practitioners and policymakers who will ensure that the project is highly relevant to end-user needs. By bringing together researchers with strong international experience and end-users with key expertise in the region, a critical mass of experience and knowledge is being mobilized to carry out the project activities.GLOBAQUA aims at addressing the fundamental need of connecting the occurrence of multiple stressors in a situation of water scarcity with the policy implementation in European river basins. The structure of the project into WPs allows sharing responsibilities between researchers who are specialists in their respective fields. The work performed by the different WPs expands through different scales, starting from the monitoring and modeling studies to the river basin scale. The achievement of an overall good status of European water bodies until 2015 (according to the WFD) poses a crucial challenge not only to water management agents but also to policy makers on different scales, the scientific community and the society in general. Overall, the synergy of the different groups arises from their different expertise that complements each other to get a holistic picture of the problem, as well as potential solutions.

516

Improving the WFD purposes by the incorporation of ecotoxicity tests and the assessment of pollutants availability

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According to the European WFD, ecological status is based on three quality elements: biological, physicochemical and hydromorphological, but ecotoxicological status is still not included. Biological status is not always in coherence with chemical status, maybe due to the adaptation mechanisms of aquatic organisms under chronic chemical exposure. In these situations, ecotoxicity tests could be useful to resolve these divergences. Moreover, the evaluation of the freshwater ecological status often supposes time, workforce expertise and costs, that sometimes the water management organisms can not assume. The application of ecotoxicity tests as preliminary investigation could help to reduce theses

disadvantages and optimize the freshwater quality studies. The objective is to design a methodology in order to study the ecotoxicological status of rivers that could be useful and complementary to ecological status by (1) comparing the effectiveness and viability of different ecotoxicity tests performed with freshwater sediments, and (2) evaluating the relationship between ecological status, pollutant concentrations, their bioavailability, and water and sediment ecotoxicity. Thirteen sampling sites within the Ebro river watershed were selected. At each sampling reach composite samples of sediment were collected. Data about priority pollutants in water, sediment and fish as well as biological and hydromorphological status and physicochemical characterization has been achieved. The ecotoxicity of pore water and whole sediments was evaluated by different bioassays (*V. fischeri*, *N.palea P. subcapitata*, *D. magna* and *C. riparius)*. The trace metals bioavailability was calculated by a sequential extraction according to the Community Bureau of Reference method. To distinguish the potentially toxic fraction associated to trace metals burden of sediments, an analysis of acid-volatile sulphide and simultaneously extracted metals was performed. The results indicate that the ecotoxicological approach in most of the analyzed sediments is in agreement with their ecological status. This study expects to demonstrate that the integration of chemical, biological and ecotoxicological analyses could be crucial to understand the hazard of pollutants in aquatic ecosystems, especially, in freshwater sediments, and it pretends to corroborate that cost effective and rapid screening short-term bioassays could be useful to determine the surface water ecotoxicological status.

517

Combined and interactive effects of three stressors (phosphorus, temperature and zinc) in a freshwater community

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During the last decades the biological and ecological responses to a suite of stressors have been examined at different levels of biological organisation (molecular to community level). However, most studies have so far focused on the effects of single stressors, while in nature multiple stressors are usually present simultaneously. At the community level very few experimental studies have investigated the combined effects of multiple stressors. Literature data suggests that significant three-way interactions of multiple stressors on organisms are common in nature. The main objective of this study is to assess the interactive effect of three different stressors on an aquatic community. An indoor freshwater microcosm experiment was conducted in which total phosphorus (oligotrophic, eutrophic), temperature (natural conditions, global warming prospective) and zinc (control, low & high) were varied in a full 2x2x3 factorial design. During the experiment general water quality (Total P, dissolved P, NO_3, NO_2, NH_4, DOC, DIC, pH, hardness, BOD_5 and O_2) and plankton abundance (phytoplankton, protozooplankton and zooplankton) community composition were recorded weekly, starting the day before the start of the treatment. At the end of the experiment the periphyton cover of the sediment was observed. Interactive effects between the different stressors on the endpoints were determined by comparing observed values with values predicted with the independent action model. The significance of the interactions (three-way and two-way) and the main effects were tested by using ANOVA's (three and two-way) on log-transformed observed data. With three-way ANOVA, both temperature and zinc were found to have effects on phytoplankton (total chlorophyll and composition) and periphyton but the zooplankton or snails only seem to be affected by zinc (at 300 μg/l of zinc all daphnia died). After 3 weeks of exposure, significant three-way phosphorus x temperature x zinc interactions were observed at every sampling for phytoplankton total chlorophyll and composition. Significant antagonistic interactions were observed starting from 3 weeks after exposure for total chlorophyll between temperature and zinc under oligotrophic conditions. Our data clearly shows that there are significant interactions between phosphorus, temperature and zinc and the different biotic communities respond differently to these stressors.

518

Cumulative effects caused by pharmaceuticals and water intermittency on algae and bacteria living in stream biofilms

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Increasing concentrations of complex mixtures of pharmaceutical compounds (PhCs) are progressively detected in many rivers, but their potential toxicity risk on non-target aquatic biota still remain poorly studied. Flow interruption is common in many river systems, and this natural stressor alters the functioning and biomass of aquatic biota and may alter their sensitivity to toxicants. This study aims to evaluate the effects of a chronic exposure of a mixture of 9 PhCs at environmental concentrations on the structure and metabolic processes of algae and bacteria communities. The study also evaluates if biofilms exposed to PhCs exposure and flow interruption have a different sensitivity than those non exposed to water intermittency. In order to address these issues, an experiment was performed using artificial streams. Biofilms accumulated some PhCs such as metropolol and hydroclorothiazide, and pharmaceutical effects were expressed on the decrease of algal biomass and cyanobacteria abundance. Also changes in the structure of bacterial community (based on DGGE fingerprint analyses) and higher rates of metabolic process (such as primary production and community respiration) occurred. In addition, the algal community exposed to water intermittency became more sensitive to short-term exposure of pharmaceuticals (lower EC_{50} value) than those growing under constant flow. These findings support that synergistic effects between water intermittency and pharmaceuticals exposure occurred on algal community. In contrast, the bacterial community previously exposed to water intermittency became more tolerant to short-term exposure of pharmaceuticals (higher EC_{50}) than those occurring under constant flow conditions (co-tolerance phenomena). This study shows that i) a chronic exposure of a mixture of pharmaceutical compounds has adverse effects on aquatic communities, and ii) that water intermittency modulates the effects of chemicals on natural communities (e.g. biofilms).

519

Assessing the effect of salinity on stream macroinvertebrate communities despite confounding factors and salinity pulses: the use of traits and large scale family turn-over.

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Licensed discharges of saline effluent from coal mines and power stations occur around the world including in the Hunter River catchment, New South Wales, Australia. Since 1994 a salinity trading scheme has largely been successful at preventing salinity levels rising above target levels of 600 and 900 μS/cm in the upper and the mid-lower Hunter River, respectively. Determining whether these levels are protective of ecosystem health in the Hunter Catchment is complicated by other sources of salinity, salinity often occurring in pulses, variation in ionic proportions of salinity and salinity concentrations being confounded with other environmental fluctuations. Here we examined changes in stream macroinvertebrate traits and large-scale turn-over of families with increasing salinity in the Hunter River and adjoining catchments (Karuah River, Lake Macquarie, Tuggerah Lakes and Manning River). Increasing salinity was found to be associated with a reduction in the abundance of salinity sensitive families ($SPEAR_{salinity}$), as indicated by laboratory tests. There was also a greater reduction in the abundance of families which were both salinity sensitive and have traits making their populations likely to recover slowly from pulse disturbances ($SPEAR_{salinity\text{-}pulse}$). These results suggest that salinity, especially pulses of salinity, are potentially altering macroinvertebrate communities, although evidence that other environmental factors may also (partly) play a role will be presented. We found that as EC increased, there was significant turnover in macroinvertebrate families including below salinity levels of 600 and 900 μS/cm. In conclusion, salinity changes in the Hunter and adjoining catchments are potentially (in conjunction with other variables) affecting macroinvertebrate community structure.

520

Community responses to the interactive effect of surfactant exposure and water depth in a microcosm set-up

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Polyoxyethylene amine (POEA) surfactants are common adjuvants in many glyphosate formulations used in Canadian forestry. Aerial application of these products can lead to exposure of small vernal pools and ponds, which do not require the protection of buffer zones. POEA has been shown to be more toxic than glyphosate alone to aquatic organisms. Additionally, the fate of POEA in the environment is not well understood. To better understand the risk posed by POEA, comprehensive exposure assessments under realistic environmental conditions are needed in order to shed light on factors such as the role of sediment adsorption or the interaction with other environmental stressors such as reduced water levels in the studied habitats. To this end, twelve microcosms (4 m diameter) with water depths of 15 and 90 cm were used to evaluate the interactive effects of depth and POEA exposure. Synthetic sediment (5% organic content) covered the bottom of each pond. Three ponds of each depth were treated with POEA at a nominal concentration of 0.520 mg/L, equivalent to the recommended application rate of 4 L/ha of VisionMax® for the 15 cm-deep ponds and three times the maximum application rate of 8 L/h of VisionMax® for the 90 cm-deep ponds. Surfactant fate, water physico-chemical parameters as well as phytoplankton (ash-free dry weight and chlorophyll a), periphyton (ash-free dry weight and photosystem II quantum

yield) and zooplankton populations were evaluated over 35 days. POEA had a short water-column half-life that was consistent with previous studies. At close-to-environmentally relevant concentrations such as the ones tested in this study, exposure to POEA did not induce significant ecological effects on aquatic communities. Depth alone had a much more significant effect on the composition and development of the studied communities. No interaction between stressors was observed. Overall, the risk posed by the POEA exposure to small, shallow pools under the studied conditions was considered minimal.

Ecosystem structures and functions and their valuation in Ecological Risk Assessment (I)

521

Defining protection goals for PPP soil ecotoxicological risk asessements based on the ecosystem services concept

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What to protect, where to protect and when to protect are fundamental questions that need to be addressed before designing risk assessment schemes. Recently EFSA has used the millennium assessment initiative and the therein specified ecosystem services (EsS) concept to design risk assessment schemes. Here we use the EsS concept to design a novel and science based proposal for soil risk assessments with PPPs. As a first step we defined registration principles for a new soil risk assessment. After defining these we identified soil relevant EsS. Thereafter, we formulated relevant protection goals which were transferred into test systems. In the next step we defined a tiered risk assessment based on acceptability criteria. The registration principles we identified were that risk assessments should be workable, scientifically sound and politically acceptable. A well-structured risk assessment would have to reduce uncertainties and it should reduce data gaps concerning organism potentially at risk. A risk assessment scheme should use validated test systems only, needs to have conservative triggers, and needs clear acceptability criteria. Soil relevant EsS (e.g. food production & nutrient cycling) can be affected by PPPs. While it is relatively easy to define general protection goals it is more challenging to define specific protection goals. In the soil area protection goals are now divided in structural and functional protection goals. In our opinion structural protection goals (protecting single species) lack a clear link to protection goals derived from EsS. Functional protection goals directly correlate to EsS. Thus tests based on functional parameters will reduce uncertainties. Although several functional tests are already available we demonstrate based on an example with a fungicide that novel functional tests are sensitive. Additionally we link these novel functional tests to structural tests. The integration of functional tests substantially improves risk assessment schemes as they have a broader scope than single species tests and are directly connected to EsS. Ecosystem services and the protection goals derived from them are suitable to design a new soil risk assessment scheme. Several EsS are relevant to soil and can be affected by PPPs. Protection goals based on EsS should be complemented by validated functional tests as these, in contrast to structural tests, will more directly address protection goals and thus fulfil registration principles.

522

Solution-focused landscape-level eco(toxico)logical assessment and management

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This contribution aims to show examples of a solution-oriented approach to environmental assessment and management at the landscape level. The case studies involve assessment of eco(toxico)logical impacts on species assemblages at the landscape scale, including ecosystem services, while having a specific focus on solving those problems. Evidence shows that impacts of man-made stressors on ecosystems vary in space and time and involve chemical mixtures as well as many other stressors affecting habitat quality, while prevention, management and restoration often rely on disparate scientific and practical efforts. Disparate in the sense of considering chemicals separate from mixtures, and those separate from other stressors, and species separate from functions, while the impact assessment is separate from finding effective solutions. The latter resulted in the proposal to improve the utility of risk assessment by generating the concept of solution-focused risk assessment. This contribution is built on eco-epidemiological examples, based on the vast amounts of (bio)monitoring data being collected under e.g. the Water Framework Directive. We sketch how these data can be used in both ecological and ecotoxicological models, and that those data can then serve to diagnose site-specific impact magnitudes and their probable causes on a landscape level. We show that mixture- and multiple stress analyses are possible, and identify the relative importance of mixtures and other stressors in shaping local species assemblages. The studies can be done for various endpoints, amongst which species diversity and ecosystem services. An example shows that management aiming at impact reduction requires a solution-focused definition of the desired status after restoration: the choice of restoration endpoint (species diversity, ecosystem services, other) is important for choices in the restoration strategy.

523

Assessment and valuation of ecosysem services: Ensuring Relevance

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The Ecosystem Service Paradigm (EsSP) can be used to define links between human activities and ecosystems; this can be used to evaluate, justify or optimize decisions. How EsS within various applications and frameworks are applied, defined, quantified, modelled, valued and communicated ranges widely, potentially hindering their roles as integrative tools. For the EsSP be useful for the cross-disciplinary integration, those in different fields must be clear about what is meant and assumed when terms are used, and within what context assessments are being carried out. Policy/action options of "consumers" should drive EsS valuation, and EsS-based assessments should be designed to link decision context to meaningful valuation approaches. The logic behind EsSP can be explained by a three-part, iterative Decision Cascade; Ecosystem Service Decision Analysis (EsSD) defines proposed policies or actions, and the changes under consideration in scenarios. Within this context, Ecosystem Service Assessment (EsSA) evaluates how such changes affect biophysical structure, and thus ecosystem function and services; Ecosystem Service Valuation (EsSV) then takes the results and generates valuations (which can, but do not need to be, monetary) to inform decisions; linking back to EsSD. EsS-based evaluations can expand the current risk-focused thinking behind ecological risk assessment (ERA) to consider trade-offs between a range of desirable and undesirable responses of a variety of ecosystem endpoints; understanding of such trade-offs is essential to inform decisions about more sustainable remediation, regulation and management. This paper describes "taxonomies" of various aspects of EsSP applications, based upon their decision context, perspective and assessment approach. Actions and policies available to "consumers" of EsSA are discussed. Then, a range of regulatory and management applications to which the EsSP can be applied are described. The manner in which EsS considerations can be used to inform decisions depends upon a range of issues; assessment and valuation approaches must be designed to ensure relevance to the specific questions and actions at hand. Both monetary and non-monetary valuation approaches can be applied, but how these values are derived, aggregated and communicated can have important implications for the relevance, transparency and sustainability of decisions they inform; these issues will be discussed.

524

Livestock activities as source of ecosystem services. A tool for Agri-Environment-Climate payments in Rural Development Programs 2014-2020. The case of the Autonomous Province of Trento.

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Agri- Environment-Climate payments (AEC) need a robust justification within the Rural Development Program (RDP). The new RDP 2014-2020 offers the possibility to include environmental benefits and externalities as relevant elements that drives decision-making. In fact, among the environmental and climate-related Focus Areas of interest for the RDP it is possible to extrapolate those ecosystem services (ES) that we think are relevant in the management of agriculture related activities. The target of AEC measures is to deliver environmental benefits efficiently: commitments based on environmental benefits must be justified by 'hard' evidence, such as case study and quantified impact models. The use of an ES platform is proposed to support, measure and account for AEC measures by employing impact models and, through scenario analysis, testing the effectiveness of the measures to be applied. The provisioning ES here considered are those related to food provision, specifically related to livestock. ES related to food provision have the peculiarity of being linked with human action. In the models here described there are in fact two components: biophysical elements and management practices. Considering the aim of the whole project, we need to include the amount and the way human action interacts with the environment. It is necessary to distinguish between the use of meadows to produce fodder crops and the use of pasture to graze livestock. One probabilistic model is built for meadows. For meadows the target is the production of fodder crops in big quantities and of good qualities: how these features are weighted depends on the sustainability degree of the practices applied. The models built for meadows and pasture act as source for other ES. This linkage allow to check the effect of more/less sustainable management practices on other ES. The assessment of variables related to management practices will in fact not only impact on fodder crop production (meadow) but also on water, carbon stock, rural landscape and outdoor recreation. The ES directly linked to grassland probabilistic model for this application are: carbon sequestration, water purification, aesthetic view and recreation. For carbon sequestration and water quality we use two specific deterministic models; for aestetic view and recreation we have built two ad hoc probabilistic models.

525

Relation between ecosystem-service perception and environmental performance assessed by LCA and Emergy accounting: a case study of pond farming in France

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In France, fish pond farming is performed on large areas with extensive practices, more of a traditional activity than a productive activity. Its continuation or development involves a variety of issues associated with environmental constraints, biodiversity support, landscape maintenance and economic return. However, no relations have been established between the environmental performance of farming systems and perception of their ecosystem services.Our study aims to clarify the link between ecosystem services perception and environmental performances of fish ponds assessed using LCA and Emergy accounting (EA). Our study was conducted in two different fishpond farming areas of France: Brenne and Lorraine. We surveyed 29 fish farms, combining its Life Cycle Inventory with a ranking of its ecosystem services as perceived by the fish farmer. In each farm, the ranking of ecosystem services was transformed into scores, and a LCA and EA were performed. Correlation analysis (using R software) was performed to analyze relations between the indicators and ecosystem-service perceptions. Perceptions of ecosystem services by fish farmers are driven by provisioning services (fish production) but also by regulation services (hydrological regulation and biodiversity support) and cultural services (especially recreation). Significant correlations ($p< 0.05$) appeared between perception of ecosystem services by fish farmers and some LCA impact categories and EA indicators. In particular, the sensitivity to provisioning services was positively correlated with eutrophication impacts and negatively correlated with the Emergy % of renewability, the sensitivity to hydrological regulation was positively correlated with land occupation and water dependence, the sensitivity to cultural services (e.g. hunting and angling) was negatively correlated with ecosystem quality, and the sensitivity to nutrient cycling service was positively correlated with ecosystem quality, water dependence and the Emergy Index of Sustainability. Most of these relations are explained by the inclusion of natural resources in the production system and by the relative attention farmers pay to provisioning services vs. support, regulation and cultural services. Thus, profiles of fish farming depending on production objectives can be drawn using the complementarity of targeted ecosystem services and environmental performances.

526

Evaluation of the ecosystem services of biocontrol and functional biodiversity in Mediterranean agroecosystems. The need for a multiple es evaluation protocol

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Cyprus is one of the most geographically isolated Mediterranean islands and a biodiversity hotspot of the world. Fourty five percent of the total island area is occupied by agroecosystems, therefore assessing ecosystem services in agricultural land is of immmense importance. Agriculture in Cyprus is characterized by both traditional low input systems such as vines as well as intensive high input systems where agricultural crops such as potatoes and tomatoes are cultivated.Agricultural intensification leads to a depletion of resources that eventually can lead to desertification. Climate change and invasive species as well as unsustainable practices are expected to further worsen desertification.A simple method using prey facsimiles was adopted in order to evaluate the ecosystem services of biocontrol and functional biodiversity within intensive tomato agroecosystems and adjacent land or field margins. The method was successful and it was shown that predation biocontrol as well as beneficial species increase five fold in adjacent natural areas compared to agricultural land, therefore its use is recommended for ES assessments in other agroecosystems. The results are discussed and the need for a multiple ES evaluation protocol is highlighted for Mediterranean and other agricultural systems.

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (I)

527

Species differences and the waltz of nanoparticle recognition

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Cells "see" the corona of biomolecules around a nanoparticle, rather than the nanoparticle's bare surface. Here we present for the first time formation, profiling and characterisation of nanoparticle-protein corona made of proteins of invertebrate origins (*Eisenia fetida* coelomic proteins; EfCP), and that this facilitated interactions with the cells from which the native proteins were harvested. We suggest that the protein corona formed of the native repertoire switches "recognisability" of the nanoparticles' biological identity and this assists the cells to interact with them. Most striking in our findings is dynamic evolution of the protein corona in the local extracellular microenvironment, where protein secretion and corona re-formation simultaneously occurred. The upshot is a three-step cycle of nanoparticle recognition, whereby 1) corona formation, 2) cellular interaction and 3) protein secretion are the key components to understand its toxicokinetics within biological systems. What appears intriguing is that such toxicokinetics of nanoparticles may not be the same even among related species of invertebrates sharing similar ecological niches, for example, between various earthworm species. This is because the protein repertoires and resulting formation of protein coronas can be species-specific. The broad implication is that the use of representative species may need careful consideration in assessing the risks associated with nanoparticles.

528

Silver nanoparticles affect the homeostasis of copper in a multi-cellular fish in vitro system

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Tight regulation of the homeostasis of essential elements is crucial for the life of all organisms. Several metal specific transporter proteins are necessary to allow this process. In this study we evaluate if citrate coated silver nanoparticles (cit-AgNPs) interfere with this complex machinery. Using lines of fish intestinal (Rainbow trout gut, RTgutGC) and hepatic (Rainbow trout liver, RTL-W1) cells this project aims at investigating the effect of cit-AgNPs on the homeostasis of essential metals such as copper, iron and zinc. To mimic more closely the *in vivo* scenario and allow polarization, intestinal cells were grown on transwells. Moreover, to evaluate the effect of cit-AgNPs following the intestinal uptake and metabolism, hepatic cells were co-cultured in the sub located well (or basolateral chamber). Cytotoxicity of silver (Ag) in its ionic ($AgNO_3$) and nano (cit-AgNPs) form was evaluated in both intestinal and liver cells. Doses of ionic- and nano- Ag that resulted in 15% reduction of viability after 24 hours of exposure were applied to the RTgutGC grown on transwells. Following these exposures RTL-W1 grown under the transwell did not show any reduction in viability. Metal analyses by ICP-MS indicated that, while intestinal cells accumulated similar amounts of silver following ionic or nano- Ag exposures, cells exposed ionically excreted significantly more silver to the basolateral chamber (containing the RTL-W1 cells). Messenger RNA levels of Metallothioneins (MTA and MTB), copper ATPases (ATP7A and ATP7B) and zinc transporter (ZnT1) were measured by quantitative PCR in RTgutGC and RTL-W1 cells. MTs mRNA levels were highly increased by both ionic and nano-Ag in RTgutGC. In agreement with the ICP-MS analyses, RTL-W1 indirectly (under transwell) exposed to ionic silver showed increased MTs mRNA levels. Moreover, the mRNA levels of ATP7B were increased in RTgutGC following both nano- and ionic- Ag exposure and ZnT1 were increased in RTgutGC exposed to ionic silver. Taken together this data suggests that cit-AgNPs enter the cells as efficiently as $AgNO_3$. However, ionic silver is excreted more efficiently basolaterally by RTgutGC cells. The increased expression of the Cu-ATPase ATP7B, which is known to transport silver, might have a role in basolateral silver excretion.

529

In vivo tracking of single walled carbon nanotubes and their modulation of nutrient transport and processing genes during fish feeding studies

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Increased use of single-walled carbon nanotubes (SWCNTs) in research and consumer industries has led to a call for reliable data on their fate and biotoxicity to better evaluate their environmental impacts and potential health effects. Previous research in our lab has shown that direct exposure of SWCNTs to the GI tract of fathead minnows via gavage did not cause overt toxicity and using near-infrared fluorescence imaging were able to determine that the SWCNTs are not likely absorbed. But the highly sorptive nature of SWCNTs may limit nutrient availability in the presence of food as they move through the digestive system under feeding conditions. To investigate whether SWCNTs can impact nutrient availability and uptake in fish, we cloned a suite of genes relevant to nutrient transport and processing (PEPT1, PEPT2, CCK, LPL) from fathead minnow tissues. We then show how these expression patterns are modulated in response to SWCNTs in feeding studies. We additionally assessed the ability of SWCNTs to interact with intestinal proteins using a quantitative ITRAQ proteomics approach. For baseline studies, fathead minnows were fed a diet consisting of ground commercial fish feed or starved for 96 hrs followed by a period of re-feeding for 96 hrs. A second study was performed where fish were given feed containing SWCNTs for 96 hrs. Result of these studies show that each generevealed a specific expression profile. For

example, PEPT1 was most highly expressed in the proximal intestine and increased in starved fish compared to fed fish. Interestingly, fish that were initially starved for a period of time and then re-fed showed that PEPT1 expression levels were similar to those observed in the fed group. Conversely, starved or fed conditions had minimal impact on the expression of CCK. These results indicate the temporal nature of nutrient-related genes in response to variable nutritional conditions. We are currently assessing the impact of SWCNT exposures on the temporal expression of these genes along the intestine and generating proteomic profiles from SWCNT-protein complexes isolated from exposed fish. Using NIRF we were able to track and quantify SWCNTs in the intestinal tract throughout the exposure periods which show the nanomaterials are primarily confined in the GI tract. The results of the current study will increase our understanding of the interaction of gut nutrients, lipids, and enzymes with SWCNTs.

530

Role of Quantum Dot Surface Functionality on Uptake and Function in Fathead Minnow Ovarian Follicle Cell Primary Cultures

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Nanomaterials (NMs) encompass a broad group of sizes, materials, and surface chemistries; raising the need to identify characteristics that are likely to influence uptake and toxicity into cells and organisms. To date, no high-throughput screening assay has been developed to identify NMs that have the greatest potential to elicit adverse outcomes on fish reproduction. Previous studies in mammalian *in vitro* systems have identified steriodogenesis as a potential target of NM toxicity. For the first time for FHM, we isolated ovarian follicle cells using a previously described method for another fish species. These primary cultures were utilized for high throughput screening of the uptake and effects of quantum dots (QDs) with different surface functionalities. Cell function was evaluated by measuring steroid synthesis/accumulation and changes in key steriodogenic genes by qPCR. We used commercially available QDs with different surface functional groups, carboxy groups (-COOH), amino groups linked to the QD with PEG (-AMINO), or PEG (-PEG) as model NMs in this study. Cells were plated and allowed to adhere for 48 hours prior to a 24-hour dosing period (10nM QDs). Following dosing the media was refreshed without QDs for a 24-hour post treatment period. At the completion of the experiment laser scanning confocal microscopy was used image cells to evaluate QD uptake. Total RNA was isolated from cells in replicate cultures for analysis of changes in STAR, CYP19A1, FSHR, and FOXL2 mRNA expression. Media from all time points and treatments was assayed for E2 accumulation. Confocal microscopy showed that QDs were internalized, and that the degree of internalization and location of NMs depended on surface functionalization. Preliminary steroid hormone analysis showed a significant decrease in estradiol (E2) accumulation in QD(-PEG) exposed cells. This is the first report of the capabilities of NMs to influence reproductive physiology of fish ovarian follicle cells. The use of high throughput screening assays, such as the assay described here, will allow for screening NMs for their cellular uptake potential as well as their ability to elicit effects on reproduction in fish. We describe here, for the first time in fish, the role surface functionality plays in QD accumulation in ovarian follicle cells *in vitro* and their effects on steroid hormone accumulation. Acknowledgement: NSF grant CBET-0853707.

531

Indium and ITO induce endoplasmatic reticulum stress

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Indium and indium tin oxide (ITO) find increasing use in numerous electronic equipment, particularly in flat screens. The unique properties of ITO nanoparticles, a sintered mixture of of indium(III) oxide (In_2O_3) and tin(IV) oxide (SnO_2), make it to a crucial compound in the electronic industry. Intruction into the environment occurs via spillage during production or leaching from electronics at their end of life, thus being part of electronic waste. However, very little is known on the (eco)toxicological profile of these metals in aquatic organsims. In the present study we assessed the molecular effects of indium nitrate ($In(NO_3)_3$) and ITO nanopaticles *in vitro* (zebrafish ZFL cell line) and *in vivo* (zebrafish embryos). To this end ZFL cells were exposed to different concentrations between 0.5 and 1000 μg/ml, while zebrafish embryos were exposed to 0.1, 1 and 10 mg/L of $In(NO_3)_3$ and ITO. Any cytotoxicity of ITO was noted up to 1000 μg/mL, whereas $In(NO_3)3$ was cytotoxic with an an EC50 of 741.3 μg/ml in ZFL cells. An ER stress response or unfolded protein response coupled with apoptosis and inflammation occurred at the transcription and protein level at concentrations of 500 μg/ml. Both the mRNA and protein levels of the marker gene BiP were induced. BiP can be released through three signalling pathways (IRE1, ATF6, PERK) under stressed conditions to enhance folding capacity. Indium results in a selective release of BiP through ATF6. Our data demonstrate for the first time that ER stress response is induced by indium *in vitro*. The toxicity of indium nitrate was much stronger than that of ITO. Similar effects occurred in zebrafish embryos, although at lower activity. However, we found a strong induction of TNF-α after exposure to 1 and 10 mg/L of both indium nitrate and ITO. The low solubility of indium in water, in which zebrafish embryos were exposed to indium is suggested to play the major role for reduced toxicity. This study unravels an unknown mode of action of indium that should be considered for the evaluation of its human and environmental health risks.

532

A comparison of toxicity and accumulation kinetics in Daphnia magna and Lumbriculus variegatus exposed to differently coated silver nanoparticles

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As nano(eco)toxicology progresses it is becoming more important to gain a deeper understanding how rates of uptake and depuration, that collectively define accumulation, may relate to nanomaterial toxicity. Understanding this is crucial to refine testing protocols and in the extrapolation of data across *phyla* for the successful implementation of nanotechnology impact assessment. Here we discuss research on two model species, *Lumbriculus variegatus* and *Daphnia magna*. Ten nm PVP, PEG and citrate coated Ag NPs, in parallel with silver nitrate ($AgNO_3$), were used. NPs were characterised as prepared and in appropriate test media. Toxicity was assessed with standard *D. magna* and *L. variegatus* static non-renewal immobility tests. Unidirectional uptake and depuration rate constants were determined using experimental principles of the biodynamic model. Different coatings lead to different acute toxicities and accumulation kinetics despite similar Ag NP core size. Test organisms showed different sensitivity and toxicity ranks to the particles. *D. magna* and *L. variegatus* acute toxicities ranged between 5-13 and 64-327μg/ L, respectively. Uptake rate constants ranged from 0.87-1.65 and 0.13-0.6 65 L g^{-1} d^{-1} and efflux rate constants ranged from 0.27-2.48 and 0.0045-0.064 d^{-1} for *D. magna* and *L. variegatus*, respectively. *D. magna* showed a biphasic depuration to all particles which is indicative of NP loss after ingestion whereas *L. variegatus* fit a 1-compartment loss model. The study indicates that biodynamic modelling of water-borne exposures can be used to explain acute toxicities and derive a consistent rank order for both species based on LA_{50} values i.e. PEG-Ag NP > PVP-Ag NP > Cit-Ag NP. However caution is required when basing assumptions on water-column concentrations as toxicity rank is not conserved across *phyla*. This may suggest physiological differences between species and specific NP-organism interactions are important determinants of bioavailability and toxicity. The data also shows it is likely Ag^+ release from Ag NPs cannot fully explain their toxicity therefore using adapted Ag^+ models to predict Ag NP toxicity may have inherent shortcomings. The authors acknowledge funding from NERC and contributions from other partners in this project. \n

Applications of innovative passive sampling and dosing (I)

533

Combining equilibrium passive sampling and dosing for determining the in situ mixture toxicity of environmental mixtures

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The advantages of passive sampling for determining the dissolved concentrations of organic contaminants are well documented – *in situ* sampling, up-concentrating of compounds with low levels, plus no need for an external power source. These same characteristics also make passive sampling an attractive option for the initial sampling of environmental mixtures prior to their introduction in toxicity bioassays. However, this approach only makes sense if the environmental mixture profile, plus the dissolved levels of the individual constituents, are preserved between the field and toxicity test. In this study, a laboratory study was performed that shows that the most reliable way to achieve these dual goals is by combining *equilibrium* passive sampling and dosing. Silicone passive samplers were exposed in model aquatic set-ups, with or without dissolved organic carbon, to constant dissolved concentrations of organic compounds of different hydrophobicities. Duplicate sets of passive samplers were removed during the kinetic and equilibrium uptake phases – with one set extracted and exchanged into DMSO for spiking, and the other group used directly in passive dosing mode. The sum toxicity measured using the Microtox assay depended on whether the passive samplers were in kinetic or passive uptake mode, and also whether the compounds were introduced by spiking or passive dosing. These differences can be explained by the variations in mixture composition and test losses depending on the approach used. Equilibrium passive sampling and dosing resulted in toxicity test dissolved concentrations that were similar to those determined in the set-ups, and thus most closely reflected the true *in situ* sum toxicities.

534

Passive sampling with POCIS for contaminants of emerging concern in

drinking water

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Contaminants of emerging concern, including pharmaceuticals and personal care products (PPCPs) and endocrine disrupting substances (EDS)have been reported in drinking water at many locations in the USA, Europe and in Canada. The source of most of the compounds is municipal wastewater. Traditional water sampling methods often require the pre-concentration of large volumes of water to detect trace levels of these contaminants. POCIS is a passive sampling technology that has been developed to concentrate trace contaminants of emerging concern in water. However, there have been few studies to evaluate whether POCIS is suitable for monitoring these contaminants in drinking water. The purpose of this study was to determine if contaminants of emerging concern are present in raw and treated water in drinking water treatment plants (DWTPs) in Ontario, Canada. Another objective was to evaluate the use of POCIS as a monitoring tool for these contaminants in drinking water. Five DWTPs in Ontario were monitored for seven "indicator compounds" that included a non-prescription pharmaceutical, two prescription pharmaceuticals, two antibiotics, an estrogen hormone and an artificial sweetener. The cocnentrations of these compounds were estimated from amounts accumulated in POCIS deployed for 2 and 4 weeks in raw and treated drinking water at the DWTPs. These estimated concentrations were compared to the concentrations measured directly from extracts prepared from grab samples of raw and treated drinking water collected at three times during the deployment period. The indicator compounds were detected in most grab samples of raw water, but detection in treated drinking water was primarily limited to samples prepared from POCIS. Comparisons between POCIS data for 2 and 4 week deployments indicated that there may have been some loss of the indicator compounds from the POCIS over the length of the deployment period. The potential for loss of contaminants sequestered into POCIS over time was confirmed in a bench scale experiment. Overall, this study demonstrated that POCIS passive sampling could be a valuable tool for monitoring contaminants of emerging concern in drinking water, but a knowledge of the dynamics of contaminant accumulation and depuration is required to interpret the monitoring data appropriately.

535

Passive sampling as a tool for monitoring pharmaceuticals and its metabolites in marine environment at trace levels

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Carbamazepine (CBZ) has been reported as a potential tracer in surface water due to its high persistence to biodegradation and poor elimination during wastewater treatment [1,2]. It has frequently been detected in wastewater effluent (up to 2.1 mg/L), in surface water (up to 1.1 mg/L) or even in drinking water (30 ng/L) [3,4]. However, little information on its detection and no data on its transformation products (TPs) in marine water are available in literature. This may be due to higher dilution rates, the complexity of the matrix (salts and ions) or/and the lack of suitable approach to detect these analytes at trace levels (ng/L). Recent studies have reported that passive samplers (POCIS) are effective samplers for qualitative and quantitative evaluation of emerging contaminants in surface water and wastewater [5,6]. In order to perform quantitative analysis it is necessary to determine the uptake rates or sampling rate (Rs) for each compound of interest in the POCIS. However, until now only some publications have reported on the application of these devices for analysis of pharmaceuticals in marine environment [1], and consequently, uptake rates have been calculated for a limited number of pharmaceuticals (approx. 25 drugs) [3,5,6]. This parameter is specific for each compound and depending on water-flow, temperature, biofouling, size of the POCIS (sorbent quantity), physico-chemical parameters (e.g., pH, dissolved organic compounds, and conductivity) as well as sampling period. Accordingly, the aim of this work was: (i) to develop and validate an analytical strategy to evaluate the occurrence and concentration of two anticonvulsants (carbamazepine and oxcarbazepine) and its related TPs in coastal water impacted by sewage treatment plant (STP) effluents; (ii) to determine the uptake rates of the target compounds under laboratory experiments; (iii) to evaluate the use of POCIS as a qualitative tool of non-target compounds (not included *a priori* in the method) from seawater.

536

Passive sampling method for the detection of pyrethroids and organophosphates in surface waters in the sub-ng/L range

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Most pyrethroids and organophosphate insecticides are very toxic for aquatic organisms (e.g. annual average environmental quality standard (AA-EQS) of cypermethrin: 0.08 ng/L). Although most of these substances are very non-polar ($logK_{ow}$ 4-8), critical concentrations can be reached in rivers. Thus, analytical methods are needed that can detect these substances in the sub-ng/L range. Gas-chromatography tandem mass spectrometry with negative chemical ionization (GC-NCI-MS/MS) has been found to be most efficient. However, there are problems during sampling, storage and extraction of water samples because pyrethroids efficiently sorb to plastic and glassware. Passive sampling on polydimethylsiloxan (PDMS) samplers can avoid this drawback and allow long sampling intervals for increased enrichment. Thus, PDMS passive samplers may be an efficient tool to detect non-polar pesticides in surface waters. In this study, a passive sampling method using PDMS sheets followed by an analytical method for 17 non-polar insecticides (15 pyrethroids, 2 organophosphates) using GC-electron ionization (EI)-MS/MS was developed, validated and applied to field samples. The PDMS sheets were extracted in a fast and efficient way using accelerated solvent extraction. Clean-up was performed using a combined silica gel and C18 column in order to reduce environmental matrix and sampler matrix. Good absolute recoveries (53–85 ± 8 %) and low LOQs of < 60 ng/PDMS were achieved for all substances. Back-calculation to time-weighted average water concentrations was done with sampling rates determined in the literature for PCBs. With this, LOQs in the sub-ng/L range could be achieved (0.002-0.13 ng/L), thus below AA-EQS values. To establish substance specific sampling rates, experiments were carried out in a flow channel system. To monitor in-situ sampling rates during field deployments, five pyrethroids were used as performance reference compounds . We generated 45 environmental samples with 2-week PDMS sheet deployment in five rivers during the main pesticide application period. Four pyrethroids (cypermethrin, permethrin, lambda-cyhalothrin, deltamethrin) and two organophosphates (chlorpyrifos, chlorpyrifos-methyl) could be detected in concentrations in the range of their AA-EQS values. The results show that PDMS samplers are very successful in detecting highly toxic non-polar insecticides in surface waters, which facilitates their future monitoring for compliance with EQS values.

537

Measurement of labile fraction of PAHs with polymeric membranes, in case of chronic or accidental pollution in rivers in France

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Over the last years, passive samplers have been used to measure the bioavailable fraction of hydrophobic organic contaminants in aquatic environments. In this paper, two main issues are addressed: (i) some methodological issues to measure the available dissolved Polycyclic Aromatic Hydrocarbons (PAHs) with polymeric membranes and (ii) the ability of polymeric membranes to measure the time-weighted average of the available dissolved concentration in water in case of variations over time (chronic or accidental pollution). To estimate the time-weighted available concentration during the exposure (TWC_{ldpe}), one first-order kinetic was used, whose the exchange kinetic parameters were calibrated in laboratory in a closed pilot. The laboratory constants were corrected from exposure conditions with the desorption of standards *in situ*. Experiments in channels bypassing the French river Gave were used to reproduce chronic pollution and accidental pollution with controlled injections of PAHs. Polymeric membranes in LDPE were deployed during 21 days in the channels. The first scenario reproduced chronic pollution by continuous injection of PAHs. The second scenario reproduced an accidental pollution by an injection of PAHs in the river during the first 3 days of the 21-day exposure. The third scenario was a discontinuous pollution with injection of PAHs during 3 periods of 3 days separated by 4 days. The same total quantity of PAHs was released in all scenario and two levels were tested: Environmental Quality Standard (EQS) level and 1/3 of the EQS level. During the experiment, grab samples of water were regularly analysed. The truly dissolved concentrations measured with polymeric membranes were compared with the actual exposure concentrations. The results have validated the laboratory calibration of kinetic parameters and the use of Performance Reference Compounds to correct *in situ* environmental exposure conditions. Polymeric membranes are overall reliable to integrate time-variations of concentrations, but the integrative performance of the tool decrease with the volatility of the compounds. To conclude, polymeric membranes are promising as alternative method to improve chemical monitoring of receiving water. They seem reliable to quantify the truly dissolved micropollutants and to integrate time-variation of concentration during pollution peak, discontinuous pollution or discharges of effluent.

Measuring and modelling chemical bioavailability in

soils (I)

538

Development of a Freundlich-type model predicting Cu, Ni, and Cd toxicity to earthworms

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This study aimed to develop bioavailability models for predicting Cu, Ni, and Cd toxicity to earthworms (*Lumbricus rubellus*, *Aporrectodea longa*, and *Eisenia fetida*) in a range of soils of varying properties. A Freundlich-type model, complying with the basic assumption of the biotic ligands model, was used to relate metal toxicity to the free metal ion activity and possible protective cations in soil porewater. LC50s based on the total soil metal concentration varied largely (usually >10-fold) in different soils. The relative sensitivity of the earthworms to metals in different soils followed the same order: *L. rubellus* > *A. longa* > *E. fetida*. A pH dependence of Cu^{2+} toxicity to different earthworm species was observed while only Mg^{2+} was found to significantly alleviate Ni^{2+} toxicity. Cd toxicity to earthworms showed to be driven by the free Cd^{2+} alone. The Freundlich-type model in which the corresponding toxicity-modifying factors were included, explained more than 84% of variations in Cu and Ni toxicity to different earthworm species. Predicted LC50s (expressed as free ion activity) never differed by a factor of more than 2 from the observed values. External validation of the model showed a similar level of precision, even though toxicity data for other soil organisms and for different endpoints were used. Although extrapolation of the Freundlich-type model across metals still needs more research efforts, this model, requiring fewer parameters than the BLM, proved to be a feasible framework for directly linking the porewater chemistry to metal toxicity in soils for various soil dwelling organisms and plants with different endpoints.

539

Implementation of bioavailability into the effects assessment of lead in soils

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At present, the risk assessment of lead (Pb) for soil in the European REACH registration file is based on a fixed generic predicted no effect concentration ($PNEC_{soil}$) for all soils. This provisional $PNEC_{soil}$ was derived from the 5th percentile of the distribution of the chronic NOEC and EC10 values for several plants, invertebrates and microbial processes. Although bioavailability and toxicity of metals can be strongly affected by the variation in soil physicochemical properties, the available Pb data did not allow establishing a relationship between soil properties and Pb toxicity to terrestrial organisms. This issue was addressed by measuring toxicity of Pb for 6 endpoints (2 plants, 2 invertebrates and 2 microbial endpoints) in a set of 7 soils covering a representative range of soil properties (pH, organic matter content, texture and eCEC). The effect of soil properties on bioavailability and toxicity of Pb was less pronounced compared to other metals (Cu, Zn, Ni, Co, Mo). Variation in soil properties only significantly explained variation in Pb toxicity for some endpoints (e.g. nitrification), but not for other endpoints (e.g. microbial respiration, *Folsomia candida* reproduction). Lead toxicity in field soils is generally significantly lower than in corresponding soils freshly spiked with soluble metal salts. This discrepancy was quantified by studying toxicity of Pb after 3 different contamination conditions: i) freshly spiked with $PbCl_2$, ii) freshly spiked, leached and pH corrected in order to remove salinity and pH stress, and iii) freshly spiked and aged for 5 years. Leaching and long-term aging of soils attenuated Pb toxicity significantly, confirming higher toxicity in freshly spiked soils compared to soils equilibrated under field conditions. Soils spiked with a lead salt to perform toxicity testing are therefore poor indicators for toxicity of Pb in soils gradually contaminated in the field and a correction factor of 4.0 was derived to correct for this discrepancy. A generic PNECsoil of 213 mg Pb/kg soil was derived as the 5th percentile of the log-normal distribution of 105 leached and aged NOEC and EC10 values, covering 27 terrestrial species or microbial processes. Normalization of toxicity data to a relevant range (10th-90th percentile) in soil properties across European soils yielded $PNEC_{soil}$ values between 171 and 442 mg Pb/kg.

540

Variability in the binding capacity of dissolved organic matters toward trace metals in soil: impact on predictive ecotoxicology

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The development of ecotoxicological models for trace metals in soil requires the critical estimation of trace metal speciation in soil solution. This estimation is notably based on the description of the binding capacity of dissolved organic matters (DOM) whose a percentage is considered to react as a “generic” fulvic acid. This percentage of reactivity is typically fixed at a unique value for all soils while experimental evidences supporting this hypothesis are rather few. The present study thus aims at exploring the variability of this percentage of reactivity for soil DOM and evaluating the impact of this parameter on the toxic effect of copper (Cu) on soil organisms as predicted with the free ion effective dose (FRIED) model. Soil solution was recovered from 55 soil samples exhibiting a very broad range of physical-chemical properties. Free Cu activity was determined analytically and modelled with the Model VII. Free Cu activity was first computed by considering a percentrage of reactivity for DOM fixed at 65 % for all soils. This consideration led to a rough prediction of the actual Cu speciation in soil solution with deviation up to 2 pCu^{2+} unit. Free Cu activity was secondly computed by fitting the percentrage of reactivity for DOM for each soil solution individually, with percentages thus ranging between 5 to 200 %. Finally, the comparison of the toxic effect of Cu on soil organisms predicted by the FRIED model was alternatively over-estimated up to 37 % or under-estimated up to 60 % when using free Cu activities calculated with the fixed percentage of DOM reactivity. These results thus suggests that the large variability in the binding capacity of soil DOM involves (i) a substantial inaccuracy in the prediction of metal speciation in soil solution and (ii) a consequent alteration of the predictive power of ecotoxicological models when considering a unique percentage of reactivity.

541

Evaluating toxicity of metals alone and in combination to terrestrial plants (L. sativa M.) by site-specific principles

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Metal toxicity is one of the principal abiotic stresses leading to potential hazardous effects to plants. Lettuce as a general eaten fresh in salads can be harmful to human health upon exposure to metals. In order to reduce the chance of metals entering the food chain, our study investigated how to identify the toxicity of common metals and their mixtures to lettuce (*L. sativa M.*) as a baseline for soil quality criteria. Hypothetical interactions with biotic ligands (BLs) and bioavailability dictated by water chemistry have been proposed to affect metal toxicity to a variety of organisms. These influences can be quantified in toxicity assessment using the biotic ligand models (BLMs). Based on the soil solution properties, hydroponic solution was selected as the test medium to mimic the ionic environment of soil. It was hypothesized that major cations in the solution would compete with added toxic metal ions for binding to the BLs. Apart from cadmium which did not present statistically significant enough to sufficiently create relationships between competing essential cations and Cd-toxicity, toxicity of the other common metals in soil was found to be obviously influenced by competing major cations (such as Mg^{2+} and H^{+}). Thus, with the known conditional stability constants obtained for copper, zinc, silver and nickel, BLMs were extended for toxicity prediction for binary metal mixtures (i.e. Cu^{2+}- Zn^{2+}, Cu^{2+}- Ni^{2+} and Cu^{2+}- Ag^{+}) by combining with toxic indexes, namely toxic unit (TU), toxic equivalency factor (TEF) and overall amount of metal ions binding to the BLs (f_{mix}). These three mechanistically based approaches all succeeded to explain mixture toxicity of common metals to lettuce. By bootstrapping method, the improvements of transforming toxic indicators were quantified. The comparison of model powers gave hints in the underling modes of action of binary-metal mixtures. The estimated coefficients were also helpful in approximately judging competitive effects of individual metal ions in the mixture and toxic potency of metal mixtures. In summary, our research expanded the usage of BLMs in assessing the potential threats of metal mixtures to terrestrial plants and broke from the limitation of TEF approach that can be only used for organic chemical classes. Keywords: metal toxicity, mixtures, biotic ligand models, plants

542

Bioavailability and bioaccumulation of copper by sediment rooted plant Myriophyllum aquaticum

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When aiming environmental risk assessment of contaminated sediments, it is essential to characterize the bioavailability of compounds by either chemical measurements or biological methods. The aims of this study are (i) to evaluate how DGT measured bioavailable copper can use to predict the bioaccumulation in the aquatic plant *Myriophyllum aquaticum* and (ii) to characterise the processes involve in the uptake. For this purpose 3 different series of experments were conducted exposing small plants with roots and whorls of *M. aquaticum* to copper spiked artificial sediments and seperately to copper spiked nutrient solution. The bioavailably copper concentration in sediments was assessed by kinetic uptake experiments using a “diffusive gradient in thin films” (DGT) sampler. Measured DGT uptake was interpreted using the DGT-PROFS model providing the diffusion coefficient of metals in the sediment and the desorption rate from weak and strong particulate sites. Furthermore, pseudo interstitial water free copper concentrations were calculated taking into account local depletion of copper close to uptake phases (DGT or plant roots). In order to characterise the kinetics of the uptake into the plant roots, a series of experiments were conducted with plants exposed directly in the nutriment solution. Experimental results can be described by a

Michealis-Menten kinetic characterised by a maximum flux (F_{max}) of 1692 ng.m^{2}.s^{-1} and a Michaelis constant (K_m) of 1200 nM. Bioaccumulated copper concentrations in the plants were compared to modelled free copper concentrations. Bioaccumulation shows a similar relationship in plants with roots when exposed to the solution or to the sediment when compared on the basis of the modelled free copper concentrations. However, no clear relationship of bioaccumulation in plants exposed as whorls as proposed by the normalised protocole and free copper concentration was apparent. Possibly, the cutting of the stem and the fact that new roots have to develop modifies the uptake processes, which appears to lead to qualitatively different bioaccumulation. The study shows that DGT measured copper concentration can be used to predict bioaccumulation in entire sediment rooted Myriophyllum plants but not in the standardised biotest using whorls. Time series of DGT measurement were necessary to model sediments properties governing the copper resupply by the solid phase and to adequately estimate the bioavailability during exposure.

Mercury Biogeochemistry and Policy (I)

543

Mercury flux from Arctic snow – Irradiation and temperature dependence

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Mercury moves to the Arctic environment via atmospheric transport, and is deposited to the surface with snow. This mercury is subject to photoreactions, which may form elemental mercury (Hg(0)) and move from the snowpack back to the atmosphere. Flux of mercury from the snowpack has been hypothesized to be dependent on incident solar radiation and temperature. Hg(0) flux from snow was measured *in situ* with coincident measurement of incident solar radiation and atmospheric temperature to quantify these relationships. Flux measurements were undertaken from March 20 to 28, 2013 in Resolute Bay, NU, Canada, using a Teflon flux chamber with a Tekran 2537 used to measure Hg(0) concentrations. A Davis Weather Station was deployed to measure air temperature every 10 minutes, and an OceanOptics USB 4000 spectraradiometer to measure incident solar radiation every 5 minutes for the duration of the study. Flux of Hg(0) from undisturbed snow was found to be linearly related to incident UV radiation (R^2 = 0.6; $p < 0.01$), while temperature may play a more complicated role. Snow from the site was collected and shipped to Acadia University (Nova Scotia, Canada) for experiments to determine temperature dependence of mercury photoreactions in snow. Snow was irradiated with 4.65 W/m^2 of UV radiation using a LuzChem photoreactor, and subjected to a range of temperatures (-2 to -20 °C). The reduction of mercury in snow was monitored using a Tekran 2537 and the kinetics of mercury reduction determined at each temperature, assuming pseudo-first order kinetics. Using the determined temperature dependence at constant UV irradiation intensity and previous work using constant temperature and varying UV irradiation intensity, a predictive model was developed for the loss of mercury from snow under given temperature and irradiation conditions.

544

Interactions between Hg in shallow aquatic environment and macrophytes

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Accumulation of Hg in primary producers is the first step of Hg uptake in food web and result in the highest biomagnification step. In shallow waters the highly toxic methyl-Hg (MeHg) is formed and macrophytes – aquatic photosynthetic organisms that can be seen by naked eyes – are the predominant primary producers playing a key role in the structure and functioning of the ecosystem. The present study aimed at investigating Hg accumulation in macrophytes, and describes the role of macrophytes, in particular *Elodea nuttallii* in Hg fate that have been identified until now including Hg cycles in sediments, methylation, bioaccumulation, and biomagnification in the food chain. Shoots of *E. nuttallii* showed the highest Hg concentrations in the Babeni reservoir –contaminated by a chlor-alkali plant. Bioaccumulation factor (BAF) in shoots of submerged macrophyte was in the range 10^5 L/kg for total Hg and MeHg respectively. Up to 28% of the Hg concentration found in this species was in the form of MeHg. N and C isotope signature suggested a role of these plants as Hg source to fish of the reservoir. High Hg tolerance and fast Hg accumulation was confirmed in *E. nuttallii*. Basipetal transport of IHg from shoots to roots was evidenced, whereas MeHg was transported acropetally only, supporting the hypothesis that IHg accumulated in shoots originated mainly from the water column, while MeHg originated from sediments and water column. At the subcellular level, 65±11% of Hg was internalized in the cytosol where it is bioavailable to herbivores, whereas 45±17% was found in cell walls. To further understand how Hg is internalized in cells of the shoot, we exposed shoots to Hg and other metals including essential (Fe, Cu, Mg, Mn, Ca, K and Zn) and non-essential metals (Cd, Ni). Only Cu resulted in a significant 97.2±0.6% inhibition of Hg uptake suggesting a putative role of Cu transporters in Hg uptake. This was further supported by transcriptome analysis which revealed a putative role of EnCOPT1 in Hg accumulation. Results suggest that *E. nuttallii* created a microenvironment favorable for Hg methylation and biomagnification. This highlights the need for an increased understanding and consideration of the role of macrophytes in the Hg biogeochemical cycle in aquatic environment and in risk assessment of this metal as shallow water represent a significant surface of our planet.

545

Benthic fluxes of mercury in a highly industrialized coastal environment (Mar Piccolo, Taranto, Italy)

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Coastal areas of the city of Taranto (Italy), have suffered from intense anthropic and industrial pressures (notably an iron and steel factory, a petroleum refinery, and the Italian Navy shipyard and arsenal) and were declared a "Contaminated site of national interest" in 1998. Previous research showed significant heavy metal contamination of the Mar Piccolo sediments, with higher concentrations in the first inlet where the Navy arsenal is located. High sedimentary Hg contents, in particular, are a cause of concern, given its possible transfer to the aquatic trophic chain and considering the widespread fishing and mussel farming activities in the area. Up to ≈ 8 µg g^{-1} of total Hg were measured in the Mar Piccolo sediments, while the concentration in seafood was below the limit established by the European Community (0.5 mg/kg w.w.) with the exception of the gastropod *Hexaplex t.*, sampled in proximity of the Navy Arsenal. As Hg is considered a priority pollutant for its toxicity, mobility, and bioaccumulation potential (especially in its organic form methylmercury), a further investigation on the role of sediments as a potential source of Hg to the Mar Piccolo aquatic ecosystem was conducted. The research was carried out in the framework of the "RITMARE - la Ricerca ITaliana per il MARE (2012-2016)" Flagship Project, financed by the Italian Ministry of University and Research (MIUR). The release of Hg from sediments was investigated by means of an *in situ* benthic chamber, as previously described for other Hg contaminated coastal areas of Italy.

546

Oxycline depth influences methylmercury production and pelagic accumulation in a small lake ecosystem

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Environmental disturbances like deforestation or climate change may influence lake thermal and oxic conditions, thereby modifying cycles of contaminants such as mercury (Hg). In a small Canadian Shield lake naturally separated into three basins, the thermocline and oxycline of an experimental basin were experimentally deepened by 4 m and 3 m respectively with a lake mixer to study the effect on the methylmercury (MeHg) production and accumulation. Passive heat transfer from the experimental basin to an intermediate basin caused a 2 m thermocline decrease in a second basin without affecting its oxycline depth. When thermocline and oxycline deepening occurred together, MeHg aqueous concentrations decreased by up to 90% in the hypolimnion, by 30 to 50% in zooplankton, and by approximately 30% in some fish species. A multiple linear regression indicated that oxycline depth significantly influenced hypolimnetic MeHg concentrations, with no significant effects of thermocline depth, anoxic water volume, nor the interface area of oxic-anoxic water and sediment area in contact with anoxic water. Fish MeHg decline varied among species, with a greater response for low oxygen-tolerant bottom-feeding bullhead compared to littoral creek chub. Increased pelagic primary and secondary production likely cause MeHg decreases in zooplankton and fish via algal and growth dilution. Environmental changes leading to oxycline deepening are therefore predicted to cause a decrease in MeHg bioaccumulation in similar lakes. If associated ecosystem impacts related to application of thermocline and oxycline deepening are deemed acceptable, this experiment provides a potential remediation method in small lakes confronted with elevated MeHg accumulation.

547

Effect of oxygen, nitrate and aluminium addition on methylmercury efflux from mine-impacted reservoir sediments

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Mercury (Hg) mines are sources of Hg pollution to downstream aquatic environments. This study presents results of laboratory sediment-water interface experiments to assess the effects of oxidant and sorbent amendments on methylmercury (MeHg) efflux from reservoir sediments contaminated with Hg as a result of upstream mining activities at the New Almaden Mining District in northern California, USA. Study sites included Guadalupe Reservoir, a 25-m-deep, 30-ha reservoir near the headwaters of Guadalupe Creek, and Almaden Lake, a 13-m-deep, 24-ha quarry lake. Study sites are located in the Guadalupe River

Watershed, a complex hydrologic system that includes six reservoirs and over 100 km of rivers and streams, south of the San Francisco Bay. In the first study, 4 chambers were collected from each site and incubated in the laboratory under aerobic conditions for 10 days followed by anaerobic conditions for 17 days. Chamber water overlaying sediments was monitored with time for a range of constituents. Fluxes of MeHg, manganese and orthophosphate from sediments were significantly lower under aerobic conditions. But sediments responded in differently in important respects. Under aerobic conditions Guadalupe sediment released total Hg, MeHg, iron and sulphate. In contrast, these constituents showed negative fluxes (uptake) in Almaden sediments under aerobic conditions. This indicates that while aerobic conditions lowered MeHg flux from sediment, aerobic sediments were a source of ionic Hg. We hypothesize that the mechanism for this observation was the oxidation of cinnabar and pyrite in Guadalupe Reservoir sediment, which being closer to the mining district, may have had more mine-related mineral ores in surfacial sediments compared to Almaden Lake. In the second study, 16 chambers were collected from the deep site in Almaden Lake. Chambers were incubated in quadruplicate under four treatments for 24 days: anaerobic control; aerobic; anoxic with nitrate addition; anaerobic with buffered sodium aluminate addition. MeHg flux from sediment was significantly lower in the aerobic treatment (0.5 ± 0.6 μg/m^2·d; mean plus/minus standard error; n = 4; flux based 10 day incubation) and the nitrate treatment (0.2 ± 1.4 μg/m^2·d) relative to the anaerobic control (17.4 ± 6.6 μg/m^2·d). However, the sodium aluminate treatment (10.5 ± 1.9 μg/m^2·d) was not significantly different from the control.

548

Methanogens as principal methylators in tropical areas

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The periphyton is considered one of the main Hg methylation sites in the aquatic environment. Methylation is attributed primarily by sulfate-reducing bacteria (SRB), however, recent studies report that other microorganisms can methylate inorganic mercury. In this context, this work has two objectives: (1) measure the periphytic MeHg net primary production rates in a tropical flooded river plain system of high ecological value, (2) Identify the main bacterial strains involved in Hg methylation by addition of metabolic inhibitors in the area. The Guapore River is located in the Mato Grosso state, and is part of the Amazon basin. Samples were taken in March 2013. A total of 160 field replicates samples of aquatic macrophytes rhizomes with their associated periphyton were sampled. Physico-chemical parameters were measured *in situ*, along with water samples for dissolved organic carbon, total nitrogen and total phosphorus. Water samples were spiked with $^{203}HgCl_2$ (~ 25,000 DPM each). Each sample group consisted of 3 replicates and 1 control, killed with 1 ml of 4 N HCl, being: (1) Control (without addition of metabolic inhibitors), (2) BESA (2-bromoethane sulfonic acid, a methanogenesis inhibitor), (3) DCMU (3-(3,4-dichlorophenyl) -1,1 dimethyl urea, a photosynthesis inhibitor) (4); molybdate (sodium molybdate, a sulfate reduction inhibitor), and (5) CC (chloramphenicol, a bacteriostatic inhibitor). Extraction was carried out using acid leaching and extraction in toluene. With no inhibitors, the primary methylation rates ($Me^{203}Hg$ as % of total added ^{203}Hg) varied between 14% and 26%, with an average of 18 %. There were no significant differences in $Me^{203}Hg$ production between lakes (p=0.232). When the net methylation rates where compared among groups, the treatments with the lowest rates were chloramphenicol (4.4B0.1), BESA (6.9т0.7), molybdate (10.6т1.6), and DCMU (12.9%т3.0). The $Me^{203}Hg$ produced in different treatments were significantly different (p = 0.001). In PCA, only the first axis resumed 90% of data variance. We found no significant relationship (Rт = 0.042, p = 0.113) between the production of MeHg and the first PCA axis.. Our study may indicate indirect contributions by autotrophic prokaryotes in Hg methylation and strongly suggests that methanogens rather than sulfate reducing bacteria were likely the primary methylators in the periphyton of our study area. **Keywords:** mercury, methanogens, radiotracers, wetlands.

Toward sustainability: benchmarks, certification and LCA (I)

549

Global Principles and Practices for Hotspots Analysis

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Hotspots analysis is a relatively new analytical tool that is being used as a pre-cursor to developing product sustainability information. It allows for the prioritization of resources and actions in industry sectors, product portfolios, product categories or individual products that really matter by virtue of their environmental, social and ethical impact profile and /or their physical trading volumes and economic sales in the economy. While hotspots analysis is proving to be a helpful and effective tool that assists in the identification of areas to be prioritized for further research or action by relevant stakeholders, there is currently no common global approach to hotspots analysis; nor has there been any effort to bring together or share best practice amongst those product sustainability initiatives currently involved in hotspots analysis. Neither do any accepted principles or guidance exist on how to translate and apply the results of this analysis into meaningful product sustainability information for use by industry, governments and other stakeholders In order to address this gap, the project will involve the organization of a global process with the following **four** over-arching objectives: To develop a common understanding of and map hotspots analysis approaches currently in use and to share the learning and experiences derived from these analyses; To develop, agree and pilot a commonly agreed methodological framework for 'hotspots' analysis approaches based on identified global principles and practices, as a means of accelerating and prioritising international action on product sustainability; To build and share the knowledge base derived from existing hotspots analysis world-wide to support the development of a richer and more global view of the product categories and individual products that offer the most opportunities for performance improvement by tackling the hotspots associated with them; and To develop and agree on a guidance document on the consistent and appropriate use of the product sustainability information derived from hotspots analysis. The presentation will provide insights into the existing challenges with regards to hotspot analysis and outline the process of how to overcome these challenges. It will make the case for stakeholders involved with hotspot analysis methodologies as well as study results to become involved in the project.

550

Creating a pilot eco-label assessment matrix based on life cycle assessment

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A growing number of different eco-labels exist, spanning a wide variety of product categories and serving different markets. While intended to represent the environmental preferability of a product they are applied to, the exact scope of environmental and social attributes that eco-labels address is oftentimes opaque to users. Whether or not the eco-label covers the most critical environmental and/or social impacts associated with a product over its life cycle is also not well understood nor communicated. In this article, eco-labels are examined from the perspective of life cycle assessment (LCA). We describe the creation of an pilot eco-label assessment matrix that seeks to analyze eco-labels' criteria against the sustainability "hot spots" of a product as indicated by LCA. A test application of the assessment matrix was conducted for ecolabels applied to two agricultural product categories: beef and cotton. These were selected, as they are known to have significant sustainability impacts. Information was gathered on known impacts for these two product categories and a basic LCA was conducted. A set of "hot spot" indicators was constructed then compared to the criteria laid out by three prominent eco-labels. The pilot testing reveals some critical gaps and differences in approach between the eco-labels assessed and the LCA results. We discuss the strengths and weaknesses of the draft eco-label assessment matrix and it's potential applicability in different contexts. The article also highlights ways in which eco-labels, LCA and LCIA data may be improved and be made more accessible. The findings demonstrate the utility of taking a structured approach to the assessment of Eco-labels serve as "trust marks", providing users with information on sustainability-related issues associated with that product, and enabling them to make a more informed purchasing decisions. **\n**

551

LCA-related benchmarking process as part of environ-mental performance assessment for buildings – a contribution to sustainable construction works

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The involvement with the issue of sustainability has led to considerable efforts also from the side of the real estate industry. As a result, an understanding of sustainability has been created and is reflected in standards such as ISO 15392:2008. Thus, a sustainability assessment of construction works includes based on the fulfillment of technical and functional requirements, the assessment of the social, economic and environmental performance. As a result of standardization activities (ISO TC 59 SC 17 and CEN TC 350) there are now available among others appropriate foundations for the selection of criteria, the calculation process as well as the data requirements for an assessment of environmental performance. The criteria were derived from the areas of protection, for whose elaboration SETAC played a significant role. By using methods of LCA, a transition from qualitative to predominantly quantitative assessment of environmental performance as an aspect of sustainability was possible. Through the publication of EPD's for construction products and the availability of databases (e.g. the Ökobau.dat in Germany) the practical applicability in the processes of planning and sustainability assessment has been improved. It is now possible the building related resource use and the effects on the environment to be calculated for the full life cycle, and be included in comparisons of variants or a sustainability assessment already during the planning phase. For the national systems of sustainability assessment in

Germany (BNB and DGNB) these calculations are an integral part. A sustainability assessment requires, in addition to the calculation of the energy and material flows, the results to be able to be classified based on benchmark values. This topic has not yet been treated in standardization. Currently the foundations for the development and application of LCA-related benchmarks for buildings are discussed. It is, among others, a question of securing the completeness of the description of the building and its life cycle, ensuring the comparability based on a description of the functional equivalent, defining the limit and target values and the development process of benchmarks (e.g. on the basis of average values or reference buildings). By performing life cycle costing as a necessary part of the sustainability assessment new possibilities arise for plausibility checks.

552

Applying PEF in practice - challenges related to the development of PEFCRs and benchmarks

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The identification of benchmarks and classes of environmental performance for certain product groups is one of the targets of the Product Environmental Footprint (PEF). Within a three-year pilotphase (started in Nov 2013), product group-specific rules (Product Environmental Footprint Category Rules – PEFCRs) should be set up and validated as well as different B2B and B2C communication vehicles should be identified and tested. The intended environmental performance benchmarks for particular product categories will describe the range in which the results could be seen as not being significantly different in comparisons or comparative assertions. In order to test the applicability of the PEF methodology and to follow up the ongoing developments in the pilot phase, especially the development of benchmarks, a case study on plastic products, specifically a comparison between bio-based and conventional plastics (Cellidor vs. PMMA) is conducted. The study includes translating existing LCA studies into a PEF – leading to 'test PEFCR' which could apply for the category 'manufacture of plastic products' (CPA/ NACE code 22.2) – and determining benchmarks and environmental performance classes for the particular product category. Regarding the development of benchmarks, as a first step the uncertainities common to the product category are analyzed. First results already indicated significant differences of up to 150 % for some impact categories due to the very different product systems of the two plastic products. As a next step, a "test-PEFCR" for the product category will be developed and five environmental performance classes will be defined, ranging from A to E with C describing the benchmark and A and E the best and worst cases. Results of this analysis, with a focus on the developed benchmarks and identified challenges will be presented at the SETAC-conference. Hence, this study helps to clarify how to deal with specification of benchmarks within the PEF methodology.

553

Agricultural production guidelines as a basis for impact assessment – a case study of Indian silk production

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The environmental impact of agriculture and textile production is of growing interest for legislators and consumers that demand environmental credentials of products and services. As but one example, the European Commission is updating the Ecolabel criteria for textiles, to inform consumers, set public procurement policies, and promote a market for green products. Because this voluntary scheme covers a wide range of products, a life cycle assessment (LCA) approach is used to detect the phases with the highest environmental impact in order to determine product specific criteria. The majority of primary production for textiles takes place in non-OECD countries and accurate data on local production practices can be difficult to acquire. Exemplifying this, despite its long history and importance as a textile, LCAs of raw silk production are lacking although preliminary results indicate high embodied energy. In order to establish a benchmark for silk impacts and identify areas with major contribution to environmental impact, we performed an outline LCA of Indian raw silk based on recommended practices and comparing it with available data on farm practices. We constructed life cycle inventories for raw silk production, categorised by production stage. Included were mulberry cultivation, silkworm rearing and silk reeling. The funcional unit is one kg of raw silk at factory gate and the impact assessment method employed was ReCIPe Midpoint (E) v.1.06, as implemented in Simapro V. 7.3.3. For the majority of the indicators, burdens are concentrated in mulberry production. When compared with other natural fibers on a mass basis, environmental impact of silk is significatly higher. Results indicate that environmental impact of guideline values and recommended practices can vary greatly, depending on the category. We argue that, while they may not be representative of the actual farm practice, LCAs based on best practice recommendations (from e.g. agricultural extension institutions) can serve as a baseline for evaluating the environmental sustainability of products for which comprehensive data is lacking or not easily accessible. This is especially relevant for silk and other products produced exclusively at small scale in low- and middle-income countries with a large proportion of impact at farm stage.

554

Comprehensive environmental sustainability assessment on Pampers baby wipes including supply chain specifics

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P&G uses Life Cycle Assessments (LCA) to learn of potential product category impacts on the environment, guide the sustainable design of products, and develop justified claims. Pampers recently innovated their baby wipes in Western Europe (WE) and North America (NA) thanks to a novel making process, allowing significant reduction in dry product weight (-19% in NA, -14% in WE). We conducted a cradle-to-grave LCA study to compare wipes from 2007 with 2013 to better understand the potential environmental impacts of the new process and optimized weight. Since the majority of relevant environmental indicators for baby wipes are driven by the production of the wipe raw materials, this innovation resulted in an improvement to all relevant environmental impact indicators ranging between 10 and 40%. Agricultural land occupation for WE is the only indicator that shows no significant change (+4%). Because life cycle impact assessments do not account for all environmental impacts, we also explored other potential environmental aspects in the supply chain. For example, sustainable forestry is not always covered well in life cycle inventory data sets. The supplier of a major wipe material achieved a total sustainable forestry certification of 75% of their fibers from tree woods. Both the smaller environmental footprint identified from LCA, and the fiber certifications, are useful checks internally and are helpful to communicate to reassure stakeholders externally. Net, this study shows that comprehensive sustainability assessments use LCA insights coupled with supply chain management and associated sustainability certification systems.

Developments in Environmental Quality Standards: bridging the gap between science and practical regulatory implementation (I)

555

Gaps in sediment quality guidelines: Incorporation of sediment physicochemical characteristics in assessing uranium bioavailability

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Sediment Quality Guidelines (SQGs) have been criticized for overlooking the important role of bioavailability and the modifying factors that control the behaviour of metals in aquatic ecosystems. However, our understanding of the factors and mechanisms controlling the bioavailability of some metals is still incomplete. In particular, there is limited information regarding the bioavailability and toxicity of sediment-associated uranium (U) to freshwater benthic invertebrates. The tendency of U to accumulate in sediments makes benthic invertebrates among the most highly exposed organisms. Benthic invertebrates are also an important group of organisms used for the evaluation of metal impacts in aquatic environments, and for the development of SQGs in Canada and equivalent criteria around the world. Where SQGs exist for U, the true risk of U exposure to benthic communities is often over- and under-predicted. Recommendations for improvement of SQGs, include the incorporation of bioavailability correction factors or consideration of the dissolved fraction as an indicator of bioavailability. It is known that sediments are important in metal sequestration and associated changes in exposure to aquatic organisms, but few studies have worked with U-spiked sediments and of those that have, only a few have used benthic invertebrates as the test species. This research focused on quantifying the changes in U bioavailability for organisms exposed to U-spiked formulated and field sediments respective to the physicochemical characteristics of the sediment (i.e., particle size and total organic carbon (TOC) content). Additionally, comparisons between U concentrations measured in the solid and dissolved phase were investigated to determine if alternatives to total contaminant concentrations in sediment could be used to better predict U bioavailability. Results from these studies have shown that with similar concentrations of U in sediment, the actual amount of U available for biological uptake is influenced by the composition of the sediment. Formulated and field sediments consisting of higher amount of clay or organic carbon content significantly reduce bioaccumulation of U to exposed *C. dilutus* larvae. This demonstrates that the current SQG approach of using total sediment concentrations as a basis for predicting U effects downstream of U mining areas, is not reliable and incorporation of sediment factors that modify U bioavailability should be considered.

556

Applicability of sediment quality standard derived using standardized equilibrium partitioning method for the assessment of water bodies' status

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In the implementation of the Water Framework Directive, quality standards (QS) may be derived in the most appropriate matrix, including sediment for e.g. hydrophobic substances which are barely detected in water. The EU Technical Guidance Document on EQS (TGD) recommends in certain circumstances to apply the equilibrium partitioning (EqP) method to derive a standard in sediment based on the standard derived in water. The TGD provides default values, in particular for Total Organic Carbon (TOC) content. This method is generally believed to be rather protective, except for highly hydrophobic compounds for which an additional factor has to be applied. This method was applied at national level in order to allow the derivation of QSsed for a large number of chemical to be investigated in an exceptional survey in which 85 chemicals were analyzed at about 110 sampling points together with TOC. The selection of chemicals to be investigated in sediment took place within a larger priorisation exercise among 2400 compounds considered as potential candidate substances; of which more than 400 are pharmaceuticals and associated metabolites, and about 70 are chemicals used in personal care and household products. The rest are mainly pesticides, biocides and industrial chemicals. The results of the monitoring campaign contributed to the selection of substances to be integrated in the monitoring programmes of the water agencies in the various river basins. Substances for which a risk of degradation of the quality of the water body was identified were prioritised. This risk was calculated using EqP method with both default and site specific values for TOC. The results show that for a significant number of stations, the estimation of the risk may be different depending of the use of default or site specific TOC. This result is substance-dependant. In addition, for those sampling sites with a TOC profile which differ significantly from the default EU sediment, the overall estimated risk is evidently more impacted. It is also to be noted that default values proposed in the TGD for the application of the EqP method at EU level are not protective in a majority of the stations investigated in this campaign. In order to increase the understanding of *in situ* toxicity, TOC as an indication of bioavailability is used to interpret the differences observed between the so-called chemical status and biological status based on biological indicators determined on the same sampling sites.

557

HBCD diastereomer levels in fish and suspended particulate matter from European freshwater and estuary sites - environmental quality standard compliance and trend monitoring

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Hexabromocyclododecane (HBCD) is a brominated flame retardant applied mainly in polystyrene foams used as building insulation, and to minor extents in flame-retarded back-coats, e.g., for upholstery textiles and in high impact polystyrene (HIPS) used in electrical and electronic equipment. An environmental monitoring project was initiated to assess the impact and relevance of emission control programmes that have been implemented by HBCD producers and users during recent year. It is designed to assess temporal trends at different sites across Europe and focuses on compartments which are expected to be sinks for HBCD (based on its physico-chemical properties and the life cycles of products containing HBCD). Samplings cover fish (bream; annually since 2007) and suspended particulate matter (SPM; every second year). Selected sites are at the rivers Tees/UK, Mersey/UK, Western Scheldt/NL, Götaälv/SE, and Rhône/FR and at Lake Belau/DE. The later was selected as site with little anthropogenic influence. For fish, annual pooled samples of usually 15 fish per site were prepared. SPM samples were collected at river sites with sedimentation traps during 4 periods of 3 months per campaign. HBCD analyses were performed applying an LC/MS-MS method that allows quantification of major diastereomers (alpha-, beta- and gamma-HBCD). Based on the results from 6 years of fish monitoring it can be concluded that at sites with diffuse exposure characteristics the concentrations of HBCD are decreasing. For example, the HBCD level of bream from the Rhône declined by about 80 % until 2012. This decrease of HBCD fish levels seems to be mirrored by a decline of HBCD levels in SPM from the Rhône site. HBCD levels of the Scheldt SPM revealed no change although HBCD levels of fish also decreased significantly. Only at one site impacted by a former point source (i.e. Tees) no decreasing concentrations could be observed so far and at one site (Götaälv/SE) an increase of HBCD fish levels was observed between 2007/2008 and 2012 (no continuous sampling at this site). In general, decreasing environmental HBCD concentrations in fish seem to be consistent with the implemented emission control measures. In the revised EU directive on aquatic environmental quality standards (EQS) for surface waters (2013/39/EU) an EQS for HBCD levels in fish was set (167 μg/kg wet weight). Fish muscle HBCD levels at all sites were below this EQS with the exception of the Tees site.

558

Identification of threshold values of accumulated metals for the protection of the aquatic ecological status. A comparison among four different species.

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Monitoring of micro pollutants in aquatic ecosystems is still mainly based on measurements in the environment (water column and/or sediment). However, these measurements only reflect the momentary pollution status and do not take into account differences in bioavailability, affected by abiotic factors such as pH, water hardness, temperature and biotic factors such as feeding habits. As a consequence, current water or sediment quality criteria for micro pollutants are not necessarily adequate and well related to effects on aquatic communities observed in the real world. Direct measurement of pollutants in biota could tackle these problems. Recent studies have demonstrated that accumulated micro-pollutant levels in some aquatic invertebrates (e.g. Hydropsychid caddisflies, zebra mussels) or levels in fish (e.g. eel and gudgeon) can be potentially used to predict ecological effects of micro contamination on aquatic communities and to derive safe pollutant levels. The aim of this study is therefore to compare the applicability of different aquatic species to derive safe tissue levels for metals in relation to ecological relevant responses. For four different species, two fish species and two invertebrate species, accumulated levels are related to effects at the community level. Selected fish species were eel (*Anguilla anguilla*) and gudgeon (*Gobio gobio*) whereas macro invertebrates were represented by larvae of the non-biting midge (*Chironomus* sp. gr. *thummi*) and the zebra mussel (*Dreissena polymorpha*).. Metal levels in eel muscle from were related to the Ecological Quality Ratio (EQR) assessed at the same site in the same period. For gudgeon we related metal levels in liver to the IBI. Metal levels in midge larvae or zebra mussels were related the Belgian Biotic Index (BBI). For most metals threshold values could be derived above which ecological quality. For example, safe levels for Cd were 0.2 μg/g dw in eel muscle but 10 μg/g dw in liver of gudgeon and *Chironomus sp.* and *Dreissena sp.*. The much lower safe level for eel can be attributed to the fact that levels were measured in muscle. We could conclude from this study that for most metals safe tissue levels could be derived that are protective for aquatic communities. However, care should be taken in the selection of the organisms. Concerning metals, fish muscle will give much lower safe levels compared to liver or whole invertebrate organisms.

559

UK fish biomonitoring highlights issues with priority substances, such as mercury and PBDEs

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Wild-caugh fish are very suitable for monitoring persistent pollutants because they accumulate pollutants over their lifetime thus: - icreasing the concentrations compared to the water and therefore improving the chance to be able to measure it - reducing the variability by integrating over a number of years - and last but not least, providing a MEANINGFUL measure of pollution with regards to risks to fish themselves and/or their predators (including humans) The usefulness of biota (fish) environmental quality standards has been appreciated in the recent revision of the EU Priority substances legislation (Directive 2013/39/EU), which includes 11 biota water quality standards (previously only 3) Already, since 2007, CEH and the English Environment Agency are building an archive of frozen fish tissue samples which will allow for retrospective monitoring in the future as well as giving an insight into the present (or recent past) chemical state of English rivers. Data from a subset of these fish will be presented on mercury, PBDEs and others The mercury EQS was exceeded in the majority of samples, suggesting some concern for predators, and a trend towards higher contamination in larger (ie. older) individuals was noticable. All fish analysed exceeded the PBDE standard of 0.085 μg/Kg for the sum of six indicator PBDEs by several orders of magnitude. The standard has, however, been set extremely low, which does not seem to be justified from the available toxicity data and would be almost impossible to monitor if the concentrations were actually that low - by contrast, the measured levels of hexachlorobenzene, and hexachlorobutadiene were always below the EQS As the Fish Archive grows it will become possible to determine temporal and spatial trends of these and other substances recent publication: Jürgens, MD, et al. (2013). "The presence of EU priority substances mercury, hexachlorobenzene, hexachlorobutadiene and PBDEs in wild fish from four English rivers." Science of the Total Environment **461–462**: 441-452.

560

Protection goal directed monitoring strategy to implement Environmental Quality Standards (EQSs) for biota

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Directive 2013/39/EU lays down biota EQSs for eleven priority hazardous

substances. Where two different standards were derived for biota, i.e., one for the protection of top predators from secondary poisoning, and another one for the protection of human health from consuming fisheries products, the lower of the two was adopted as the overall quality standard. This practise may cause difficulties in implementation of the biota standards, as different protection goals require different monitoring strategies. Therefore, we propose a protection goal directed monitoring strategy. For protection from secondary poisoning, the strategy includes (1) identifying the relevant fish-eating top predator, (2) examining prey composition and size range, (3) sampling a 'prey basket' and (4) analysing the priority substances in whole fish. For protection of human health from consumption of fisheries products, a different approach has to be applied. In that case commercially important local fish species need to be sampled and the filet analysed. In a pilot study, mercury concentrations were determined in muscle and whole fish samples from three fish species collected at Lake Großer Stechlin, a reference site some 70 km north of Berlin, in September 2013. The data were used to evaluate the impact of the selected species or 'prey basket', the selected length range, and the analysed tissue on the assessment of compliance with the biota EQS for mercury. Furthermore, we will present results from mercury monitoring in fish conducted within WFD monitoring programmes in Germany by the competent authorities of the Federal States and will evaluate these data in view of compliance assessment and the suggested protection goal directed monitoring strategy. Our results will feed into the work of the EU group drafting a guidance document on chemical monitoring in biota with focus on compliance checking, which was recently established under the Common Implementation Strategy for the WFD.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (I)

561

Multi-generation study - reproductive effects of 17β-dihydroequilin, estrone, and 17β-estradiol in Japanese medaka (Oryzias latipes) following combined protocols OECD 229 and OECD 234

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Two separate studies were conducted to understand the endocrine effects of 17β-estradiol and Conjugated Estrogens (CE), specifically, estrone (E1) and dihydroequilin (DHE) on three generations of Japanese medaka. In the multigeneration life-cycle test, medaka were exposed to nominal concentrations of 1.0, 7.5 and 75 ng a.i./L each of E1 and DHE, for total exposure of 2.0, 15 and 150 ng a.i./L in combination, a positive control of 100 ng a.i./L E2 and a negative control. Two additional dosing groups were exposed; one to 1.0 ng a.i./L E1 alone and the other to 1.0 ng a.i./L DHE alone. Following a pre-exposure period of 26 days to identify actively spawning fish, spawning groups of 5 males and 5 females were formed and exposed for 21 days under flow-through conditions to evaluate reproduction of the parental generation (F0) as per OECD 229, Fish Short Term Reproduction Assay (FSTRA). Following the FSTRA, an evaluation of sexual development in the F1 generation using eggs from the F0 generation carried through 83 days post hatch (dph) was conducted as per OECD 234, Fish Sexual Development Test (FSDT). Following the 83 day exposure of the F1 generation, most of the treatment groups were terminated in keeping with the FSDT. However, exposures in the 2.0 and 15.0 ng a.i./L E1/DHE combination doses, as well as the negative control were continued through reproduction (22 days) and hatching of the F2 generation embryos (4 dph). A similar study was conducted to evaluate 17β-estradiol at nominal concentrations of 2.75, 8.8, 28.2 and 90.1 ng/L. Survival, growth, reproduction (fecundity and fertility), hatching success, sex ratio and VTG data will be presented for these three endocrine disrupting compounds.

562

Proposal of how to update the standard information requirements in REACH, PPPR and BPR – a testing strategy for identification of endocrine disruptors

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This presentation is based on a project prepared by the Danish Centre on Endocrine Disrupters (CEHOS) for the Danish EPA. The project aim to provide a science based input to the on-going work in EU with regard to endocrine disruptors, i.e. the development of criteria for identification, REACH review on EDs and the revised strategy for the future work on endocrine disruptors, focusing on adequate detection of substances with endocrine disrupting properties under various legislative frameworks, including REACH (EC No 1907/2006), the Plant Protection Products Regulation (PPPR) (EC No 1107/2009) and the Biocidal Products Regulation (BPR) (EC No 528/2012). There are currently no specific information requirements or testing strategies with regard to endocrine disruption in REACH and other relevant legislations. However, in relation to biocides and recently also to plant protection products, indications of endocrine disrupting properties of a substance trigger additional information/testing requirements. With regard to plant protection products a new regulation from 1 March 2013 (EU 283/2013 (active substances) + EU 284/2013 (products)) sets out the general data requirements from 1 January 2014. According to these, new test methods that include endocrine sensitive endpoints have been included with regard to human health and the environment. Similar data requirements and new test methods that include endocrine sensitive endpoints are included in the guidance on Regulation (EU) No 528/2012 on how to fulfil data requirements for biocides. To a limited extent in vivo test methods that include endocrine sensitive endpoints have also been included in the standard information requirements for substances regulated under REACH. The existing information/data requirements in REACH, PPPR and BPR are not sufficient to adequately detect substances with endocrine disrupting properties. Proposals for changes in the existing REACH, PPPR and BPR information/data requirements are therefore provided. The proposals include enhancement of standard test methods as well inclusion of new methods and recommendations are given for a testing strategy. For all substances (prioritized by tonnage and exposure scenario), QSAR studies and testing using in vitro assays for interaction with different ED modalities e.g. ER, AR and steroidogenesis interference, should be conducted to elucidate whether there are alert(s) for further testing for ED effects.

563

Mind the gap: Concerns using endpoints from endocrine screening assays in risk assessment

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Endocrine *in vivo* screening assays not only provide mechanistic information on the potential of a substance to interact with the endocrine system, but also data potentially relevant for risk assessment. However, these screening assays have a number of limitations that should be considered before the direct use of such data for risk assessment purposes. This paper discusses the limitations that should be considered for both human and environmental risk assessment. A proposal is made to provide an objective and transparent process in order to consider which endpoint(s) might be incorporated into a risk assessment, and when more definitive studies may be of value. Such an approach is necessary to ensure the appropriate use of screening data, and to enhance our understanding of the (eco)toxicological profile of substances undergoing screening.

564

MTBE and endocrine disrupting potential: An hypothesis-driven weight of evidence analysis

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Methyl tertiary-butyl ether (MTBE) is a fuel component used in gasoline production to increase combustion efficiency and bring associated environmental benefits, with some minor specialty uses as a solvent in closed systems. Impacts on endocrine system endpoints have been noted in several repeated exposure rodent toxicology studies using very high doses of MTBE. In addition, a study performed with zebrafish suggested that MTBE might be estrogenic. Resolving whether or not MTBE should be considered an endocrine active compound is important given increasing worldwide concern about endocrine disrupting substances accumulating in the environment and their potential for causing adverse effects on public health and wildlife. Evidence is presented for/against the idea that MTBE has potential to interact with key endocrine pathways in mammalian and non-mammalian organisms. A weight-of-evidence (WoE) analysis was conducted, focusing on specific hypotheses related to disruption of estrogen, androgen, and thyroid hormone pathways, steroidogenesis and also specifically aromatase, the enzyme that converts androgens to estrogens. This WoE procedure involved systematic consideration of each endpoint observed in one or more study designs and a semi-quantitative weighting of relevance of each type of endpoint to a given hypothesis to reach scientifically justified conclusions based on the totality of the evidence. Studies performed at extremely high doses in rodent studies in some cases confound the identification of direct endocrine system responses. However, maximal use of all existing relevant and reliable information and consistent observations in multiple studies increase support for or against a given mode of action (MoA) hypothesis. While the MTBE database continues to grow and its evaluation is ongoing, a systematic and transparent WoE assessment of the results from a wide variety of mammalian and fish studies suggest that MTBE is not primarily impacting endocrine pathways.

565

Do concentrations of ethinylestradiol and estradiol in European rivers exceed proposed EU environmental quality standards?

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This study used a geographic based water model to predict the environmental concentrations of 17a-ethinylestradiol (EE2) and 17b-estradiol (E2) throughout European rivers. The work was prompted by the proposal of the European Community (COM(2011)876) to consider these chemicals as candidates for future control via environmental quality standards (EQS). National drug consumption information, excretion, national water use, and sewage removal rates, were used to derive per capita sewage effluent values for the European countries . For E2, excretion rates of the natural hormone and national demographics were also included. Incorporating this information into the GWAVA model allowed water concentrations throughout Europe's rivers to be predicted. The mean concentration from the expected sewage discharge scenario indicated that 12% by length of Europe's rivers would reach concentrations greater than the proposed 0.035 ng/L EQS for EE2. For several countries, between a quarter and a third of their total river length would fail such an EE2 EQS. For E2, just over 1% by length of rivers would reach concentrations greater than the 0.4 ng/L proposed EQS.

566

Investigation of Health Effects Associated with Indirect Potable Reuse Water

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Population growth and urbanisation drives unquenchable demand for ever larger volumes of clean safe drinking water. In some countries suitable sources of drinking water are becoming so scarce that they will not be able to satisfy future demand. In 2007, the Southeast of England was classified as experiencing 'serious' levels of water stress. Consequently, there is a need to find alternative sources of safe drinking water that can be used for potable supply or to augment current sources. Advanced water treatment methods are now being examined to investigate whether treated domestic sewage effluent can be treated to drinking water standards and discharged near a drinking water abstraction point; a process known as Indirect Potable Reuse (IPR). Technological solutions to address the water sustainability problem must be shown to remove biologically active chemicals, including endocrine active chemicals, to levels below which they no longer pose discernible hazards to human health. In this presentation, we outline new research on the health risks associated with exposure to Indirect Potable Reuse water using developmental assays in zebrafish. We show that short-term developmental assays in zebrafish combined with genomic analysis (microarrays) can be used to identify potential health outcomes associated with exposure to chemicals present in treated sewage effluent before, during and after advanced treatment. When applied to IPR, this approach can be used to determine the extent of treatment needed to remove chemicals to levels where developmental and/or genomic effects associated with potential health outcomes compared to the control water are no longer seen. Such information is needed to inform decisions about the application of advanced treatments that could be used to ensure the sustainability of potable drinking water.

Reduction, Replacement, and Refinement (3Rs): Animal alternative approaches in ecotoxicology and risk assessment (II)

567

An alternative testing strategy for neurotoxicity using zebrafish

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Recent epidemiological studies have indicated that exposure to low doses of environmental contaminants during human development can have deleterious effects on cognitive development in childhood. The EU project DENAMIC aims to develop better and sophisticated tools, procedures and testing methods to screen compounds for (developmental) neurotoxicity and to improve assessment of exposures and effects (more information on www.denamic.org). As part of the project, a new alternative assessment strategy is under development to prioritize compounds for further in vivo rodent testing. To this end, hazard characterisation of pesticides and environmental pollutants at the molecular and cellular level is carried out, with emphasis on adverse effects during neuronal development. Test chemicals are selected based on their environmental relevance, known neurotoxicity and different modes of action. Experiments are performed with acute or subchronic exposures. Zebrafish larvae are used as an alternative animal model to study neurotoxicity in vivo. To this end developmental toxicity as well as altered behaviour is monitored. Compounds inducing behavioural effects are then studied in more depth to investigate the underlying mode of action. We have developed a new testing regime to better understand behavioural effects in zebrafish and link them to toxic mode of actions. A subset of the investigated chemicals is selected for further in vivo testing in mice and rats to validate the alternative assays.
Behavioural tests in rodents are the standard test used for neurotoxicity testing. This study will ultimately demonstrate the usefulness of zebrafish embryo assays as an alternative testing strategy for developmental neurotoxicity testing.

568

Development of an In Vitro Approach to Assess Disruption of Steroidogenesis in Native Fish Species

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There is concern regarding exposure of aquatic organisms to chemicals that interfere with the endocrine system. One critical mechanism of endocrine disruption is impairment of steroidogenesis that can lead to altered or delayed sexual development, and ultimately reproductive failure. To enable objective risk assessments of environmental contaminants, it is critical to identify the sensitivity of species native to the environments in question. However, to date little is known about the sensitivity of fish native to northern ecosystems to disruptors of steroidogenesis. One of the main challenges with assessing the effects of contaminants to native species of interest is that they are often difficult to culture or maintain under laboratory conditions. *In vivo* studies to characterize endocrine effects are also labour, resource and time intensive. Furthermore, some of the species of interest are endangered or threatened in their ecosystems. The aim of this study was to develop an *in vitro* gonadal explant assay enabling the assessment of endocrine disrupting chemicals on sex-steroid production in wild fish species. Northern pike (*Esox lucius*), walleye (*Sander vitreus*), and white sucker (*Catostomus commeroni*) were sampled from Lake Diefenbaker, Saskatchewan, Canada at pre-spawn and multiple post-spawn time-points. Gonads were excised and exposed for 24 hours to a model inducer (forskolin) and inhibitor (prochloraz) of steroidogenesis. Concentrations of 11-ketotestosterone (11-KT) and estradiol (E2) in plasma and media were quantified using ELISA. Tissues exposed to forskolin showed a concentration dependent increase in 11-KT and E2. Exposure to prochloraz resulted in a decrease of 11-KTand E2. Results illustrate that the gonadal tissue is undergoing steroidogenesis in an *in* vitro setting that is reflecting reproductive seasonality, and is also responsive to chemical exposure in a concentration-dependent manner. White sucker were found to be the most responsive species, with the seasonal time-point of greatest sensitivity differing between sexes. When exposed to 3 μM forskolin, white sucker males and females produced up to 15-fold greater 11-KT and 15-fold greater E2, respectively, when compared to the solvent controls. In conclusion, seasonality of reproductive function represented a critical factor that needs to be considered when using this here-established *in vitro* explant assay to assess responses of native species to disruptors of steroidogenesis.

569

Physiologically based toxicokinetic models for in vitro-in vivo extrapolation of receptor-mediated effects in rainbow trout

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The European REACH regulation was established by the European Parliament and the Council to prospectively avoid the negative effects of industrial chemicals on humans and the environment. To meet this mandate, the chemical industry must provide toxicological and ecotoxicological data, potentially requiring a large number of animal experiments. To minimise the use of animals, however, the 3R principle (i.e., reduction, replacement, refinement) demands that animal experiments should be substituted with appropriate alternative test methods whenever possible. Many dossiers submitted to the European Chemicals Agency for the authorisation of chemicals have attempted to provide the required data without performing new experiments, relying heavily on *in silico* methods (e.g., quantitative structure-activity relationships, read-across, grouping or weight-of-evidence approaches); *in vitro* assays were scarcely used. Here, we propose a methodology that uses physiologically based toxicokinetic (PBTK) models to extrapolate *in vitro* data to the *in vivo* level. We collected experimental results for *in vitro* and *in vivo* ethoxyresorufin-*O*-deethylase (EROD) and vitellogenin (Vtg) induction in rainbow trout (*Oncorhynchus mykiss*) following chemical exposure (either via the aquatic exposure medium or intraperitoneal injection) and compared those results with model predictions. We found that the predictive power of aqueous chemical concentrations was limited; median effect concentrations (EC50s) based on internal concentrations in fish correlated far better with *in vitro* EC50s (97 % of the values deviated less than one order of magnitude from the regression of *in vivo* and *in vitro* data). The present study demonstrates that PBTK models can be used to ordinate important biochemical markers of mechanism-specific effects (i.e., EROD and Vtg) to correlate with experimentally derived *in vitro* data. We believe that the presented non-experimental *in silico* methods for *in vitro-in vivo* extrapolation have the potential to achieve an overall reduction in the number of toxicological and ecotoxicological experiments with live animals when combined with experimental *in vitro* test methods while maintaining an equal level of protection.

570
A 3D spheroidal fish liver model alternative to better understand bioaccumulation in ecotoxicology
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The use of three-dimensional cell culture models (spheroids) are becoming more widely used in toxicological research, particularly in the fields of pharmaco-toxicology, cancer and food research. This is due to their ability to simulate the *in vivo* environment more effectively than standard *in vitro* models, which lack the 3D micro-environment of intact tissue.We recently developed and characterised a method to produce primary hepatocyte spheroids from rainbow trout that provides long term cultures. This new model has the potential to pre-screen xenobiotic compounds and measure whether they can be metabolised by fish. While *in-vitro* systems do not fully replace the requirement for *in vivo* studies, they can be useful adjunct models with a higher throughput that can support the ecotoxicological evaluation of the potential risk of aquatic pollutants, offering an alternative to animal testing. Spheroid models offer additional functionality over conventional *in vitro* cultures, in particular defined *in-vivo* like tissue architecture and good cell-cell and cell-matrix interactions are longer-lived. As such they can be developed to offer a new *in vitro* screening tool for environmental toxicological monitoring. Here we report the development of a Loss of parent assay used to determine the rate at which parent compounds were lost from spheroid cultures over a period ≤ 72 h and monitored the appearance of some primary metabolites. Compounds were selected on the basis of their physicochemical properties, existing comparable datasets and known toxicity profile. The system was fully characterised and validated with the non-selective β-blocker propranolol to establish a positive control compound and the rate of metabolism K_{met} values were calculated for all compounds. Our results compare well with data from the literature and demonstrate a current predictivity of ~70 %, suggesting that this new model is worthy of further dedicated study for its application in accumulation studies. Keywords: 3Rs, bioaccumulation, alternatives cell culture\n\nAbstract type: Poster

571
Predicting bioconcentration of benzo-a-pyrene in fish based on different fish cell lines
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Quantification of chemical toxicity continues to be based mainly on measurements of external exposure. Yet, in order to understand, interpret and extrapolate toxic effects, using internal concentrations of chemicals is more suitable. Though these can be measured, being able to predict them would be much more efficient and feasible. Predicting chemical internal concentrations accurately is, however, still challenging as biotransformation processes can strongly influence the extent to which chemicals accumulate in fish. For this reason, toxicokinetic models, which link *in vitro* biotransformation data to the whole organism bioconcentration of chemicals, have been developed. However, these models thus far are based on the assumption that biotransformation in the body solely occurs in fish liver. Since this is unlikely to be true, this assumption may result in underestimating the biotransformation of chemicals in fish. In our study we measured biotransformation in rainbow trout cell lines of liver (RTL-W1), intestine (RTgut-GC) and gills (RTgill-W1). Acknowledging that the liver is the main site of biotransformation whereas gills are not, we assumed that the gill cell line can represent cells also from other richly perfused tissues. We distinguished the intestine and pyloric caeca from richly perfused tissues in order to account for biotransformation occurring in the intestine as an important environment - organism barrier. The highly hydrophobic (log K_{OW} = 5.99) polycyclic aromatic hydrocarbon, benzo-a-pyrene (BaP), served as model chemical. It had previously been shown to be quickly biotransformed by fish which resulted in over 1000-fold differences in measured versus predicted bioconcentration factors (BCFs). The biotransformation of BaP in all cell lines was incorporated into the Physiologically Based Toxicokinetic (PBTK) model for rainbow trout. All applied fish cell lines biotransform BaP; however, the biotransformation in gill cells took more time than in other cell lines. BCF estimated by our PBTK model assuming biotransformation only in liver, was around 3000. However, taking into account also biotransformation in other tissues based on cell lines data reduced BCF further to 1000. This value is very similar to measured BCF (920). Thus, building a virtual fish based on different cell lines can help improve predicting BCFs of highly biotransformed compounds.

572
Passive and active uptake of pharmaceuticals by a primary fish gill cell culture model
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Traditionally, whole organism studies are used to assess the bioconcentration of compounds from the water. If we are to replace or reduce the large numbers of fish used in these regulatory tests then an effective and realistic *in vitro* screen is required. The gills of fish are continuously bathed in water and are a principle site of both xenobiotic uptake and efflux. The current study uses an *in vitro* rainbow trout primary gill cell culture system, which forms a polarised epithelium with high transepithelial electrical resistance (TEER; >20 KΩ cm^{-2}) and low paracellular permeability that tolerates apical freshwater exposure as *in vivo*, to assess pharmaceutical uptake and efflux. Bidirectional studies of seven pharmaceuticals (propranolol, metoprolol, atenolol, formoterol, terbutaline, ranitidine and imipramine) showed that all were transported across the epithelium in both directions. Concentration equilibrated conditions with equivalent concentrations of drugs on both sides of the epithelium revealed the active transport of propranolol and imipramine from the apical to the basolateral compartment. Further assessment of the non specific β_1/β_2-adrenergic antagonist, propranolol, revealed at very low environmentally relevant propranolol concentrations (ng L^{-1}) transport is saturable before higher concentration exposures show increased passive uptake and a concentration dependent rate of transport. Known inhibitors of drug transport proteins (e.g. SLC22, 47, SLCO, ABCBs and ABCCs); cimetidine, MK571, cyclosporine A and quinidine inhibited propranolol uptake, whilst amantadine and verapamil were without effect. Together, our results suggest that there is passive transcellular uptake of the natural form of propranolol that is enhanced by water, and the active uptake of the cationic form is perhaps via OCT2 and an MRP transporter. The uptake and efflux of propranolol *in vitro* using this gill cell system correlates with predicted *in silico* and measured *in vivo* plasma concentrations, further validating the use of this model as a potential complement or surrogate to refine, reduce or replace the numbers of fish used in bioconcentration studies.

Ecosystem structures and functions and their valuation in Ecological Risk Assessment (II)

573
How widespread are effects of organic toxicants on ecosystem functions?
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The decomposition of allochthonous organic matter and primary production are key ecosystem functions that provide energy to aquatic ecosystems and are therefore crucial for ecosystem services of freshwater ecosystems. Organism groups involved in these processes include invertebrates, bacteria, fungi, macrophytes, phytobenthos and phytoplankton. Studies addressing the effects of organic toxicants on these ecosystem functions are relatively scarce, but several investigations have been conducted focusing on effects on the involved organism groups. Our talk targets two questions: At which concentrations may ecosystem functions be affected? How widespread are potential effects? To evaluate the thresholds at which effects at ecosystem functions may occur, we reviewed existing studies on the effects of toxicants on ecosystem functions. In addition, we derived effect thresholds for the different organism groups that are involved in the functions (e.g. invertebrates, primary producers) based on a literature survey. These thresholds were then used to evaluate the concentrations of organic toxicants in surface water in the European Union using data from more than 4,000 monitoring sites. We found that more than one third of studies on the effects of organic toxicants on ecosystem functions indicated reductions in ecosystem functions at concentrations that are assumed being protective in the regulatory context. The reduction in allochthonous organic matter decomposition was more pronounced in the presence of invertebrate decomposers compared to studies where only microorganisms were involved. Moreover, organic chemicals were likely to exert acute lethal and chronic long-term effects on sensitive invertebrate and algae species in 16% and 42% of the 4,000 monitoring sites, respectively. Pesticides were the major contributors to the overall chemical risk. The risk increased with the number of ecotoxicologically relevant chemicals analysed. As most monitoring programs only included a subset of these chemicals, our assessment still underestimated the actual risk. We discuss several aspects of the relationship between structural effects and their consequences for ecosystem functions. In addition, we highlight research needs for future studies on ecosystem functions. We conclude that organic toxicants are occurring widespread in concentrations that affect organisms involved in critical ecosystem functions.

574

Effects of fungicides on leaf decompostion in vineyard streams
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Organic matter decomposition (OMD) is central for river ecosystem functioning. However, pesticides can impair this function in agricultural streams. Only a few studies have examined the effect of fungicides on OMD, and if so mainly in the laboratory. In this study we quantitatively analysed the effects of fungicides on decomposer communities and OMD in a German vineyard area. Exposure to polar organic pesticides (16 fungicides and 4 insecticides) was evaluated during four different rainfall events using passive samplers (Empore™ SDB-RPS disks), and the required calibration information was generated in a microcosms experiment. Event-driven water samplers (EDS) were also collected for comparison with the time weighted average concentrations (TWA) provided by the passive samplers. Sampling sites were located in river reaches along a gradient of fungicide exposure, and leaf litter bags were deployed for two weeks. Monitoring included also inorganic copper in stream sediments, decomposer communities (macroinvertebrates, fungal biomass, fungal diversity and bacterial density) and physico-chemical variables. The two fungicide sampling systems were in good agreement for most compounds. TWA mean and maximum concentrations for individual compounds ranged from 0.01 to 0.18 μg/L and from 0.02 to 1.72 μg/L, respectively. EDS concentrations exceeded TWA concentrations on average by a factor of six. Fungicide exposure led to changes in the structure and composition of fungal and macroinvertebrate communities. A decrease in fungal biomass and an increase in bacterial density were found along the increasing exposure gradient. Despite these changes translated to a detectable decrease in microbial OMD rate, we did not find an impact on macroinbertebrate OMD rate. This may be explained by the abundance of gammarids in most of the sites regardless of the fungicide pollution. Our main findings were that passive sampling is suitable to quantify episodic exposures from polar organic pesticides and that functional redundancy in microbial decomposer communities do not buffer effects of fungicides on microbial OMD.

575
Hyporheic zones are important for the attenuation of pharmaceuticals – myth or fact?
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Hyporheic zones are well recognized for their contribution to processing of nutrients and organic matter in streams. With respect to organic micropollutants, the role of hyporheic zones for the attenuation of these contaminants has yet to be defined. In this contribution, we will present a compliation of field and laboratory studies that allows a thorough and critical evaluation of the role of hyporheic zones in the attenuation of organic micropollutants. The environmentally relevant pharmaceutical diclofenac will be used as an illustrative example. Three experimental approaches were used in the laboratory: 1) static water/sediment tests along the lines of the of the OECD 308 guideline; 2) recirculating sediment columns that provided more defined and controllable advective exchange and redox conditions; and 3) experiments in a bench-scale flume where the complex mixing between surface water and the hyporheic zone can be simulated. In the field, we carried out mass-balancing studies at two German wastewater-impacted rivers. The results from the laboratory experiments provide clear evidence that hyporheic sediments have the potential to biotransform many pharmaceuticals. In flume experiments, the diclofenac dissipation time (DT_{50}) ranged from 3 to 9 days, depending on the experimental conditions. The two more simplified test systems provided useful for determining upper and lower threshold values of the DT_{50}. The field studies showed an efficient elimination of diclofenac in one river, while it was not attenuated in the other river. This is in contrast to findings from the laboratory experiments where the elimination was similar in sediments from both systems. The overall comparison of our studies shows that we are currently lacking methods to transfer results obtained in the laboratory to the field. This is predominantly caused by a lack of methods for quantifying hyporheic exchange on the scale of at least 10-20 km. This constitutes the major knowledge gap that prevents converting lab-derived transformation rates into reach-scale elimination rates of pharmaceuticals and other organic micropollutants. Overall we conclude that hyporheic attenuation of pharmaceuticals can indeed be an efficient elimination pathway in rivers. But favorable hydraulic conditions are required and at rivers with limited hyporheic exchange even a high transformation potential in the hyporheic zone does not provide substantial elimination of pharmaceuticals from the surface water.

576
Actively use sediments in groundwater pollution management
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At various sites in the Netherlands further migration of contaminated groundwater plumes is prevented by seepage of contaminated groundwater to surface waters. During this discharge in surface waters, the redox conditions in which the contaminations are present undergoes a change from anaerobic to aerobic, and the contamination passes a sediment layer (especially sludge) at the bottom of the surface water. These processes are favourable for natural degradation of several contaminants like chlorinated ethenes and BTEX. This decontamination process is an important ecosystem service that can actively be used in pollution management. In the cities Amersfoort, The Hague and Haarlem groundwater polluted with chlorinated ethenes already seeps to surface water. It is expected that natural attenuation processes occur in the sediment. In order to gain insight in which layer the capacity for biodegradation is present, different sediment layers have been sampled at the sites. In the sediment samples both chemical analyses and molecular analyses have been performed. Our research has shown that in general in most studied sediments within the flowpath of the pollution the capacity for biological degradation (both reductive dechlorination and aerobic degradation) is present. However, the activity of the ecosystem service depends on the thickness of the active layer because this determines the size of the 'in situ bioreactor'. This has implications for the dredging managent in this specific canal. Dredging of the canal will, at least temporary, decrease the dechlorinating capacity as shown. This causes an increase in chlorinated ethenes in surface water which, at high incoming concentrations, might result in ecological risks during a certain period of time.

577
Assessing river bank filtration with micropollutants
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A 2012/13 study of AWEL (Zurich State Office for Water, Energy & Air) investigated on the contamination risk of micropollutants from 7 waste water treatment plants (WWTPs) in the perialpine River Glatt valley, Northern Switzerland, to River Glatt (average flow rate at 2 sampling stations: 4 and 8 m3/sec) and to ad-jacent ground water (8 drinking-water wells) of an alluvial sand-and-gravel aquifer (hydraulic conductivity ~80 m/day; groundwater flow velocities 1 - 5 m/day). River Glatt loses continuously water along much of its reach. The ground-water study focused on benzotriazole, carbamazepine, sulfamethoxazole, and the artificial sweetener ace-sulfame. These contaminants revealed groundwater concentrations in the range of ng/L, ex-cept for ace-sulfame (μg/L; 1 – 2 orders of magnitude higher than those of carbamazepine). The con-cent-rations in the ground water did not vary significantly throughout a one year's period. Compared to those in the WWTP outlets, fractional con-centrations were at less than 15 per cent. Carbamazepine and ace-sulfame were considered mobile and persistent in the studied subsurface. Fractional bank-filtrated water was cal-cu-lated from concentrations and mass flow rates of these two contaminants. Values were found to be in the order of 50-70 per cent. The mixing equation allowed us to quantify further the infiltration mass flow of river water to ground water, for various realistic scenarios, including bank-filtration rates from flow-meter measure---ments, and the assumption of zero acesulfame contribution from ground water not of bank filtration origin.

578
Invertebrate traits and trace metals in the impact assessment of an exceptional river reservoir flushing
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The Integration of the functionality of ecosystems and their services into Ecological Risk Assessment is one of the main challenges in current ecotoxicology. There is growing evidence that threshold values obtained in laboratory are not protective of ecosystem function in the field. Our study explored the impact of the flushing of a run of the river reservoir. We studied trace metal concentration and speciation and the consequences on biodiversity and species traits of benthic macro-invertebrates in a secondary channel. The response variables included taxonomic richness and a diversity index (rarefied richness). The affinity of the individuals in the community to certain traits (eg. percentage of individuals in the community breathing with gills) was compared before and after the event. Suspended particulate matter concentrations increased up to 80 times during the flushing. In consequence, the concentration of metals bound to SPM increased as well. In the dissolved fraction, there were increased concentrations of Al, Mn, Co and Ni while Cu showed a clear decrease. Both taxonomic and rarefied richness decreased after the event. The trait profiles did not change, indicating that there was no measurable impact of the flushing. This led to the conclusion that the use of traits to study exceptional events has to be further investigated and that it is important to consider several, complementary response variables.

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (II)

579

Surface functionality dependent toxicity of graphene nanomaterials in the nematode Caenorhabditis elegans: A systems ecotoxicology approach

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Graphene is a single-atom-thick sheet of sp2-bonded carbon atoms in a closely packed honeycomb two-dimensional lattice structure, isolated from its three-dimensional parent material, graphite. As it has great potential for wide range of application due to attractive physicochemical properties, their possible environmental health and safety impact raised considerable concern. In this study, the potential hazard of graphene nanomaterials, was investigated in the nematode *Caenorhabditis elegans* exposed to graphene oxide (GO) and reduced graphene oxide (rGO) using an integrated systems toxicology approach. We first compared the toxicity of GO and rGO to *C. elegans* at the organism level, using survival and reproduction as endpoints and found that GO led significant decrease of reproduction potential. To understand the mechanism of differential toxicity, we conducted microarray followed by pathway analysis on GO and rGO exposed C.elegans. We tried to link GO-induced gene expression to reproduction decline using the *C. elegans* functional genomics tool. The pathway that popped up from the microarray and pathway analyses was selected and loss-of-function mutants of these genes were exposed to GO and rGO and their responses were compared with that of *wildtype*. We demonstrated that the distinct oxidation states regulate the nature of dispersibility of GO and rGO and in turn their nano-bio interactive effect which was reflected from differential toxicity. Here we combined toxicogenomics - pathway analysis with functional genomics tools based on *C. elegans* mutant libraries, which enhance and complement the predictive power of toxicogenomics and pathway analysis. This systems-based integrated approach is particularly valuable for investigating the toxicity of new chemicals, whose mode of action is not fully understood or incompletely characterized, such as with graphene nanomaterials, since it provides a comprehensive insight into the mechanism of toxicity.

580

Highthroughput genomic and proteomic effects of silver nanoparticles in tissues of the marine ectothermic mollusc Mytilus galloprovincialis

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Marine mussels show optimal features to assess ecotoxicological effects of nanomaterials due to their filter feeding behaviour, biogeographical distribution and ecological position within coastal and estuarine environments. In this study we used a combined highthroughput genomic and proteomic approach to analyze the effects of silver nanoparticles and silver ions in tissues of mussels challenged for 28 days day in microcosm exposures. Specimens of M. galloprovincialis were maintained in 10 L microcosms, fed algae daily and exposed to silver nanoparticles (5 nm, paraffin coated, Amepox, P) and silver nitrate at nominal levels of 0.2 10^-3 mg/L ; 2.0 10^-3 mg/L and 20.0 10^-3 mg/L per day. Specimens falling within same level (LOEL) for scope for growth were used for further analysis (20.0 10^-3 mg/L for silver nanoparticles and 2.0 10^-3 mg/L for AgNO3). Transcriptomic (pathways) analysis in digestive gland showed the involvement of genes with a role in heavy metal homeostasis (ferritin and metallothioneins), molecular chaperons (mainly HSPs), mitochondrial functions and different proteolytic pathways for either silver forms. A higher implication of cytoskeleton and intracellular part (membrane-bounded and non-membrane-bounded organelles) related genes was observed for the nanosilver form. Main results obtained so far by means of immunoblot analysis of 2D gels have showed increased protein oxidation (cysteine carbonylation) and protein ubiquitination in the digestive gland tissue of the 5 nm nanoparticle treatment class. Furthermore, enhanced heat shock activity was recorded in same tissues as judged by means of western blot using polyclonal anti-HSPA1A antibodies. The analysis of tissues from silver nitrate exposed specimens is currently under investigation. Our results showed the occurrence of overlapping and specific molecular responses due the challenge of Ag ions and metal nanoparticle. We conclude that common molecular traits between the two silver forms (i.e. homeostasis, oxidation and protein breakdown) are be due to the chemical reactivity of Ag ions which can eventually dissolve from nanoparticle while different traits should depend on the different way silver is presented, imported and further handled into the cell. *Acknowledgement*- This research was granted by CP-FP 247739 NanoFATE

581

A microarray and qRT-PCR study on the effects of metal-containing nanoparticles (CuO, CdS and Ag) on mussels M. galloprovincialis.

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Nanotechnology is a rapidly growing field which involves research and development of nanosized materials. Among them, metal-containing nanoparticles (NPs) such as CuO, CdS and Ag NPs, are widely used in different industrial processes and thus may finally reach the environment. However, little information is available on the behaviour of nanosized materials in aquatic environments and potential toxicity to aquatic species. The study of alterations in gene transcription levels could provide an early warning of the potential toxicity of NPs due to the rapid response of genes to environmental alterations. With the aim of assessing transcription level effects caused by metal-containing NPs in aquatic organisms, mussels *Mytilus galloprovincialis* were exposed to uncapped CuO (10 μg Cu/ l), glutathione capped CdS (50 μg Cd/ l) and maltose capped Ag (0.75 μg Ag/ l) nanoparticles (CuO NPs, CdS NPs and Ag NPs, respectively) for 1 and 21 days. A 8x15 custom mussel microarray containing 2900 identified genes and about 3000 unknown sequences was used and transcription levels of several target genes was studied by quantitative Real Time PCR (qRT-PCR) using TaqMan® or SYBR®Green methods. Based on LIMMA statistical analysis (not adjusted p-value< 0.001), different transcription patterns were found depending on the NP used: 42 different genes were regulated after 1 day of CuO NP exposure but only 4 were regulated 21 days after, including the defensive agent *fucolectin 6*. CdS NPs significantly regulated 69 different genes at day 1 of exposure and 25 genes at 21 days, being among them genes coding for metal ion binding proteins, such as metallothioneins, and proteins involved in the immune system, in protein degradation and in apoptosis. Ag NPs significantly regulated 20 different genes at day 1 and 13 genes at 21 days. Transcription level of most of the significantly regulated genes measured by qRT-PCR showed the same trend as in microarrays. Overall, transcription patterns were differently altered depending on the type of NP and exposure time. Alterations were more marked after 1 day of exposure, suggesting a successful adaptation to exposure conditions. Funded by EU 7th FP (NanoReTox, ref CP-FP 214478-2), Spanish Ministry of Science and Innovation (NanoCancer CTM2009-13477), Basque Government (Consolidated Research Group GIC IT810-13) and UPV/EHU through the Unit of Formation and Research "Protection of Ecosystem Health" (UFI11/37).

583

Interactive effects of n-TiO2 with other contaminants on immune and digestive gland function of the marine bivalve Mytilus

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The increasing use of manufactured nanoparticles-NPs (e.g. in industrial applications and consumer products) will lead to their release into the aquatic environment, where NPs will mix and interact with other common environmental pollutants, such as organic xenobiotics and heavy metals. Hence the importance of studies directed towards elucidating the interactive effects of NPs and other co-existing contaminants, possibly leading to changes in toxicity and/or bioconcentration. Suspension-feeders have been shown to represent important test organisms to evaluate the biological impact of NPs. However, data on the interactive effects of NPs with other pollutants in marine organisms are still scanty. The effects and mechanisms of action of different types of NPs heave been widely investigated in the model marine organism *Mytilus*; these studies underlined the importance of the immune and digestive gland function as main targets for the impact of NPs in marine invertebrates. Here recent data on the effects of n-TiO_2, one of the most widespread type of NPs in use, in combination with TCDD and Cd^{2+}, chosen as models of common and persistent organic and inorganic contaminants, respectively, on *Mytilus galloprovincialis* will be summarized. The results demonstrate that combined exposure to n-TiO_2 and other pollutants exerted mainly antagonistic and synergistic effects on both immune and digestive gland biomarkers, depending on type of contaminant and the endpoint measured. These data reveal complex and often unexpected interactive responses of NPs with other pollutants from the molecular to the organism level, as well as differences in bioaccumulation, in marine bivalves.

584

Interaction of engineered nanoparticles with PAHs: Could they act as "Trojan-horses" to induce biological responses in marine species?

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Little is known about potential impact of manufactured or engineered nanoparticles

(ENPs) either alone or in combination with other environmental contaminants on aquatic species. Using marine bivalve: *Mytilus sp.*, we assessed the biological responses following exposure to C_{60} fullerene (C_{60}) and two different model polycyclic aromatic hydrocarbons (PAHs): fluoranthene and benzo(a) pyrene (B(a)P). An integrated approach, which included determination of 'clearance rates' (at individual level), histopathological alterations (at tissue level) and DNA strand breaks (comet assay: at cellular level) was adopted to determine the potential impact. Transcriptional alterations of a range of genes involved in DNA damage and stress responses and in classical signalling pathway (i.e. *cyp4ya, jnk, mdm, bax, rad51, p53, ras*) were also determined by real-time quantitative PCR in tissue specific manner for the assessment of impacts following C_{60} either alone or in combinations with PAHs. Combined exposures with fluoranthene and B(a)P provided conflicting results for 'clearance rate' and DNA strand breaks, in terms of whether the responses were additive or not. Consistent histopathological abnormalities were however clearly found in selected organs (i.e. adductor muscle, digestive gland and gills) for both exposure scenarios. For the exposure to B(a)P and/or C_{60}, a significant induction for transcriptional activation of genes involved in classical signalling pathway showed tissue and chemical-specific pattern with large tissue-specific and inter-individual variations. Formation of DNA adducts however could not be detected for any exposure conditions. Overall, biological responses at different levels showed variable sensitivity. This integrated approach could be used to determine the impact of ENPs with other environmental toxicants in the complex environment.

Applications of innovative passive sampling and dosing (II)

585

Passive sampling of organic ions with ion exchange membranes - Application for the determination of freely dissolved concentrations in protein binding experiments

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Equilibrium dialysis has been used as a standard method to study the sorption of organic compounds to suspended or dissolved materials such as humic matter, phospholipid liposomes and proteins. Because dialysis experiments have several disadvantages, many alternative methods have been developed. A novel tool for the determination of the freely dissolved concentration in aqueous suspensions is passive sampling with polymeric materials. Due to limited availability of convenient sorption materials only a few attempts have been made so far to adapt this technique to ionic compounds. The aim of this work was to test the applicability of ion exchange membranes (IEMs) for passive sampling experiments with organic ions. IEMs are expected to have various advantages for passive sampling of ions such as high affinity and permeability for ionic compounds having the opposite charge. In this study the sorption properties of two types of IEM (a cation exchange membrane for cations and an anion exchange membrane for anions) were characterized, and their applicability for the measurement of the freely dissolved concentration in binding experiments with bovine serum albumin (BSA) was investigated. For experiments three anionic and three cationic compounds with different ionisable groups were chosen. IEM-water partition coefficients and the equilibration time with the IEMs were determined. Equilibrium between IEM and water was reached quickly within a few hours for the anionic compounds and within 1 to 3 days for the cationic compounds. Binding isotherms to IEMs were measured for three compounds and were found to be non-linear. Furthermore, the sorption to the IEMs was independent of pH, but highly influenced by the salt concentration of the medium. BSA-water partition coefficients were investigated with the passive sampling method and, for comparison, also with equilibrium dialysis. Both experiments yielded very similar results. Additional tests indicated that the ion exchange sites of the IEMs are hardly filled by BSA. Overall, the IEMs were found to be a useful passive sampling material for organic ions. The main advantages of the passive sampling with IEM were the high enrichment of the test compounds in the IEM and the short equilibration time for anionic compounds. The non-linear sorption of the test compounds to the IEMs and the dependence on the salt concentration of the medium may be critical factors that need attention.

586

Application of passive dosing to the evaluation of toxicity of reactive hydrophobic organic chemicals

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Passive dosing is an emerging technique that controls free concentration of hydrophobic organic chemicals in a desired experimental system. Various successful applications include the determination of partition coefficients and water solubility of sparingly soluble chemicals and the evaluation of toxicity of single chemicals or mixtures. However, the majority of studies on the evaluation of toxic potential of hydrophobic organic chemicals aimed at the evaluation of baseline toxicity or receptor binding that can be explained by equilibrium speciation. Because a constant exposure condition for highly hydrophobic chemicals can be easily achieved by passive dosing, its applicability could be extended to the evaluation of toxicity of chemicals that undergo metabolic transformations. In this presentation, we introduce cytochrome p450 family protein 35A gene expressions of *Caenorhabditis elegans* exposed to chlorpyrifos as an example application of the ecotoxicity of hydrophobic chemicals at lower doses. Gene expression results obtained using the conventional spiking method passive dosing. A simple toxicokinetic model that includes metabolic transformation of chlorpyrifos was also proposed to explain the apparent gene expression of *C. elegans*. Because metabolic transformation of chlorpyrifos is regulated by P450 family genes, it was assumed that metabolic transformation rate is proportional with the increased gene expression. The proposed model qualitatively explained the observed gene expression under two experimental conditions although it is needed to measure body residue concentration for further validation of the model. In conclusion, passive dosing would be a powerful technique when combined with sensitive markers such as gene expression that involve chemical transformations.

587

Is equilibrium sampling applicable in routine sediment monitoring?

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Freely dissolved concentrations (C_{free}) of hydrophobic organic contaminants in sediments are considered to be the effective concentrations and are more indicative of potential exposure of aquatic organisms than total concentrations (C_{total}). Passive equilibrium sampling approaches can be used to measure C_{free} in sediment pore water. Thereby, glass jars with silicone coatings of few μm thickness are very convenient for routine monitoring campaigns since the risky and time-consuming equilibration is done in the laboratory. Tedious time-serious measurements are avoided by incubating sediment sub-samples in coated glass jars with different thicknesses of silicone for validation of equilibrium sampling. Though the German Federal Institute of Hydrology has regularly monitored sediments from the German part of the River Elbe for total concentrations of, e.g., polychlorinated biphenyls (PCBs) and Dichlordiphenyltrichlorethane (DDT) and their metabolites since the 1990s, C_{free} in these sediments are largely unkown. Coated glass jars were applied to sediments from the River Elbe in order to measure C_{free} of PCBs as well as DDT and their metabolites. For this purpose, sediments were sampled at ten stations within the German part of the River Elbe from the Czech border to the wire near Geesthacht. Sediments were incubated in silicone coated glass jars for two weeks in the laboratory, the silicone was extracted and analysed by GC/MS/MS detection. Analyte amounts in silicone were proportional to the amount of silicone for all investigated contaminants and sampling sites confirming equilibrium sampling. C_{free} were in the pg / L range for PCBs and up to the lower ng / L range for DDT metabolites. Patterns of PCB accumulation in equilibrium samplers with highest values primarily for PCB 138 and PCB 153 were similar to C_{total} quantified by traditional exhaustive extraction and analysis. Though, C_{total} of p,p'-DDT are high in the River Elbe, p,p'-DDT was rarely detected in equilibrium sampling extracts and could only be quantified in sediments from two stations hinting at a strong binding of p,p'-DDT to sediment particles. In comparison with other DDT metabolites, C_{free} of DDD isomers were highest. Overall, C_{free} of PCBs and DDT metabolites clearly reflect the contamination of the river Elbe. For PCBs, estimated concentrations in biota ($C_{sed, lipid}$) obtained by equilibrium sampler data highly correlate with bioaccumulation in fish.

588

Coastal Bothnian Sea sediments as a source of dissolved PCDD/Fs and PCBs to water

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The aim of this study was to investigate whether coastal sediments in the Bothnian Sea act as a sink or source of dissolved PCDD/Fs and PCBs to bottom water. High levels of organic contaminants in Baltic Sea biota have been a matter of great concern during the last decades. Concentrations of PCDD/Fs and dioxin-like PCBs in lipid-rich fish of the northern parts of the Baltic Sea (Bothnian Sea) exceed the EU quality standard for food and feed of 4 pg toxic equivalents (TEQ) /g fw. Sedimentation of particulate organic matter is the most important removal process for PCDD/Fs in the Baltic Sea. We used polyoxymethylene (POM) passive samplers to measure the freely dissolved concentrations of PCDD/Fs and PCBs in pore water and bottom water in eight different areas along the Swedish coast of the Bothnian Sea. The study areas represent various ongoing and historical industrial activities. We also investigated the importance of local sediment contamination for observed concentrations of PCDD/Fs and PCBs in stationary juvenile perch caught

in the same areas. Activity Ratios (calculated from concentrations in pore water and bottom water based on chemical activity ratios) for PCDD/Fs were higher than one at all stations (average 27; stdev 22) implying higher fugacity in the sediment and that the sediments have a high potential to act as a source of dissolved PCDD/Fs to the water column. Activity Ratios for PCBs varied between 0.3 and 17(average 2; stdev 4). The concentrations of PCDD/Fs and PCBs in bottom water correlated positively with concentrations in juvenile perch. We know from recent studies that actions have to be taken to reduce atmospheric transport and deposition of PCDD/Fs to reduce levels in the Baltic Sea in general and in herring specifically. Still, the results presented here suggest that contamination of PCDD/Fs and PCBs in the lower trophic levels of the aquatic food web in Bothnian Sea coastal areas may be strongly affected by contamination in sediments. The results from this study thus imply that continued efforts to reduce the levels of PCDD/Fs and PCBs in coastal sediment will have positive effects on concentrations of these contaminants in the lower trophic levels of the coastal ecosystems.

589

Initial laboratory validation experiment: comparing the accumulation of organophosphorus flame retardants in passive samplers and mussels (Mytilus edilus) during continuous exposure.

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A number of field studies have been conducted in recent times using passive samplers in the aquatic environment and more recently integrated assessments using both biomarkers and passive systems. In this study a flow through dosing system was used to expose mussels (*Mytilus edilus*) and silicon rubber passive samplers to relevant organophosphorus flame retardants (OPFRs) which are largely recognised as priority hazardous substances in the marine environment. The experiment ran for a total of 28 days and included a depuration period during which the concentration decline in both samplers and mussels was monitored. The flame retardants with the higher K_{ow} values generally accumulated to a greater extent in the mussels and the passive samplers. For the majority of OPFRs tested there was faster uptake in the samplers but during the depuration phase there was a higher loss from the mussels most likely due to metabolism. However TPrP despite having a low K_{ow} was detected in the mussels all be it at a low concentration. The following compounds were below detection limits in the mussels but were present in the samplers: TCEP, TCPP and TBEP and all had a log K_{ow} value < 4. Based on these results it appears that the samplers could be more appropriate to use when there are pulsed or episodic environmental exposures as they are more likely to be detected. In general mussels are the preferred monitoring tool when the assessments involve food-chain effects and give a more realistic indication of actual exposure and effects and likely levels of contamination within organisms. As integrated field monitoring using both biomarkers and samplers increases laboratory derived data like those produced during this experiment will become ever more important to ensure that the appropriate methods are used to monitor accumulation of contaminants in the marine environment. Currently we are investigating the use of these samplers post exposure in ecotoxicological assays using other relevant species for example marine algae to assess toxicity of environmentally relevant concentrations.

590

Legacy Persistent Organic Pollutants Have Reached the Remoteness Place on Earth: the Antarctic Plateau

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The Antarctica is usually perceived as a symbol of the last great wilderness and remoteness. Although natural "barriers" such as oceanic and atmospheric circulation protect this region from lower latitude water and air masses, previous assessments on concentrations of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) evidence its ubiquitous presence in air, snow, water, vegetation or food webs organisms in the Maritime Antarctica, thus, the outermore region of Antarctica. The occurrence of these man-made synthetic chemicals in Polar Regions is just another manifestation of the multiple anthropogenic perturbations on the composition of the biosphere. Their long half-lives facilitate repeated cycles of volatilization and deposition resulting in progressive movement away from temperate and tropical source regions towards colder climate areas and remote regions. Ultimately, these compounds may experience "cold-trapping" at the Polar Regions, where the colder temperatures further prolong persistence and enhance their accumulation in snow. In addition, this cold trapping in the outermost regions of Antarctica could prevent or retard their transport to the Antarctic Plateau. The extreme persistence, semi-volatility, biacummulation potential and adverse effects of some POPs in wildlife and humans have led to develop international protocols regulating their use (Stockholm Convention). Most of the studies available in the antarctic atmosphere reporting data of PCBs and OCPs were mainly performed using active samplers mostly located close to research stations since power supply is usually a limiting factor. This limitation together with the extreme conditions of performing an atmospheric sampling campaign over the Continent result in a lack of information regarding the levels of PCBs and OCPs, or any other POPs, over the Antarctic Plateau. Therefore the main objectives of this work are: i) to assess for the first time, in an Antarctic Expedition, the occurrence of PCBs and OCPs over a latitudinal gradient, from Novolazarevskaya Station (75 km from the coast) to Glaciar Union crossing the South Pole during 35 days (total 3500 km) using a passive sampler coupled to an exclusively wind-power kite-drawn sled and ii) to prove if the eco-friendly vehicle developped by Larramendi and moved with big comets could be a useful a tool for adventorous Antarctic research.

Measuring and modelling chemical bioavailability in soils (II)

591

Effect of various cations in phosphate-based washing solution on toxicity of washed arsenic-contaminated soils

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Arsenic contamination of the smelting area has been a major concern, as it could impose adverse impacts on the surrounding ecosystem. Soil washing using phosphate-based reagent is often used to remove heavy metal from soils as a more environmentally-friendly alternative than acid-based washing. Although phosphate can effectively remove arsenic from soils through phosphate-As competition ion exchange, the residual heavy metal after soil washing or the soil washing procedure itself that could deteriorate the soil qualities could still impose toxic effects on the ecosystem when the treated soils are put back. The aim of this study was to determine the toxic effects of the As-contaminated soils before and after laboratory-scale phosphate-aided soil washing treatment. The As-contaminated soils was collected from the former Janghang smelter site in Korea. The contaminated soil samples were collected from two different locations (A and B) of the site. The soil samples from the location A were sand with 33.2±10.8 mg kg^{-1} of As, while the soil samples from the location B were silt loam with 31.1±14.5 mg kg^{-1} As. Two soil samples was mainly contaminated with As, and other heavy metals were also found in soils. For soil washing, 0.5 M ammonium phosphate ($(NH_4)(H_2PO_4)$) is to be used at the soil to solution ratio of 1:5 for 2 h, which was determined based on the preceding study results. The Microtox® assay using *Vibrio fischeri* was performed to determine the toxic effects of the soils before soil washing and the toxic efects after soil washing are to be determined. From ammonium phosphate-aided soil washing results, about 24% of As was mainly removed in both of soils located from site A and B, showing most of soils achieved the concentration level provided by Korean Act. However, Microtox® results showed that the toxic effects could not be correlated to the As concentration in soil samples, suggesting that other heavy metals could have effect on toxicity of washed soils. It is expected that the toxicity of washed soil caused by the other heavy metals can be reduced by competition with cations in phosphate washing solution. Thus, soil washing using the other phosphate-based solution such as KH_2PO_4, $Ca(H_2PO_4)_2$, and $Mg(H_2PO_4)_2$ will be also conducted to compare the toxic effect of washed soils with ammonium phosphate washed soils. Based on this result, the most suitable phosphate-aided washing solution is to be selected for the site application.

592

In vivo and in vitro tests of the bioavailability of dioxins and polycyclic aromatic compounds in field-contaminated soil

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Contaminants present in soils are only partially available for biological uptake, due to sequestering or strong sorption to soil particles. However, the methodologies for taking bioavailability into account during risk assessment of contaminated sites are not established. In this study, four different tests of the contaminant bioavailability were applied to soils from two contaminated sites. The contaminants investigated were polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs, also known as dioxins), and polycyclic aromatic compounds (PACs). PACs includes polycyclic aromatic hydrocarbons (PAHs), but also more polar PACs such as the oxy-PAHs and azaarenes. These substances can be formed in the same processes as PAHs, but can also be produced as a result of PAH transformation in the environment. The oxy-PAHs and the azaarenes are generally more polar than the PAHs, and may therefore display a different behavior at contaminated soils, for instance in bioavailability and leachability. In this study, the seventeen 2,3,7,8-substituted dioxins, 16 PAHs, 11 oxy-PAHs and 4 azaarenes were included, thus covering a wide range of substances of importance at sites contaminated with organic

pollutants. Four tests were used to investigate the bioavailability of the contaminants. These were two *in vitro* tests – extraction using Tenax and extraction mimicking human gastrointestinal uptake (oral bioaccessibility) – and two *in vivo* tests – uptake by the worm *Eisenia fetida* and by the zucchini *Cucurbita pepo*. The aim of the study was to determine the bioavailable fraction and investigate how it varies over the contaminated sites, how well the results of the different tests correspond to each other, and to what extent the bioavailability according to the different tests correlates to soil parameters such as organic carbon, black carbon, nitrogen and phosphorous.

593

Integrating mechanistic modelling in estimating metal accumulation in plants

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Vegetable consumption is the most important contributor to human body burden many metals. In available human exposure models, metal concentrations in the edible parts are empirically estimated from the total concentration in root, using Bio-Concentration factors or regression equations. A mechanistic method, consisting of three steps: speciation, root uptake modelling, and root-to-shoot translocation modelling, was developed to assess metal accumulation in plants. Firstly, metal concentrations in soil solution were estimated from total metal concentrations in soil using multi-surface complexation models (speciation). Uncertainties in this step are induced by lacking data on soil properties. Secondly, metal accumulation in roots was estimated using the WHAM-HA modelling, i.e., humic acid was considered a surrogate to roots. Uncertainties in this simulation are associated with the wide variations in equivalent site density of roots. Thirdly, root-to-shoot translocation was modelled to predict metal concentrations in the above-ground parts of plants. The translocation depends on shoot growth and xylem loading, which are driven by passive diffusion or mediated by transporters. These factors are, in turn, influenced by plant characteristics. Therefore, plant characteristics are the most important source of uncertainties in this step. When data on soil properties were available, the estimates of soil solution concentrations were generally within one order of magnitude of the measurements. Based on generic soil properties, such deviations remained for metals for which their sorption can be simulated well by intensive data in the models such as Cu only. Higher uncertainties were seen for others, e.g., four orders of magnitude for Ni. Based on the available value of the equivalent site density, the predicted total concentrations of metals (i.e., Cu, Ni, Mn, Cd, and Zn) were in general within one order of magnitude of the measured values. The variations in the total root concentrations equal the variations in the equivalent site density of plant roots (a factor of 2). The estimates of shoot concentrations were within one order of magnitude of the measurements. Metals are mainly retained in roots while only a small amount was transported to the above-ground parts.

594

An in silico model of ingestion bioaccessibility for selected polycyclic aromatic hydrocarbons in coking works soil using gene expression programming

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A new in silico predictive model has been produced for in vitro human ingestion bioaccessibility of heavy molecular weight (HMW) polycyclic aromatic hydrocarbons (PAH) (≥4 ring) using gene expression programming (GEP). GEP is an evolutionary algorithm that searches for patterns in data and expresses them as transparent symbolic regression equations. Findings suggest that GEP can be used to identify meaningful complex relationships between soil properties and PAH resulting in the production of a site-based predictive model for the bioaccessibility of HMW PAH. The identification of these relationships indicates that physico-chemical properties of soil and PAH are important determinands of bioaccessibility at the site which may be used to develop further research hypotheses. The in silico model is based on twenty-nine input variables for sixty-four soil samples from a former coking and chemical works located near Chesterfield in Derbyshire, UK. Samples were freeze dried and sieved to ≤250 mm prior to quantification by GC-MS for parent PAH (sixteen defined by the United States Environmental Protection Agency as priority pollutants) and bioaccessible concentrations of HMW PAH, extracted using the FOREhST method. Near infra-red (NIR) and mid infra-red (MIR) diffuse reflectance spectroscopy (DRS) was completed on all soil samples to determine their chemical bonding characteristics. Principal component analysis of the raw MIR and NIR data was used to create a sub-set of model input variables. Total organic carbon and pH were also determined given their influence on contaminant sorption. The completed dataset was randomly partitioned into training and testing data (70:30) and used for GEP modelling. Evolved models were evaluated using visual summaries (scatter plots and residual plots), goodness-of-fit metrics (mean percentage absolute error, R^2 and relative root mean squared error) and one-at-a-time response function sensitivity analysis. The preferred model shows good agreement between measured and predicted data, emphasising the applicability of the in silico approach to predicting the bioaccessibility of HMW PAH using selected physico-chemical soil properties. The model is not supposed to be a replacement for existing in vitro extraction methods, but instead a site-specific complementary tool for human health risk assessment of land contamination.

595

Use of diffuse reflectance mid-infrared spectroscopy for rapid prediction of physico-chemical properties of sediments

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The rapid and inexpensive determination of physico-chemical properties (e.g. particle size, cation exchange capacity (CEC), total organic carbon (TOC)) that mitigate the toxicity of both inorganic and organic contaminants in sediments could be useful for assessing the "susceptibility" to contamination. Techniques based on diffusive reflectance mid-infrared Fourier-Transform (DRIFT) spectroscopy have been successfully used for the prediction of many soil properties. This study describes the development of partial least squares regression (PLSR) models combining DRIFT spectra and reference analytical data for the prediction of sediment properties, relevant to determining contaminant toxicity. A total of 79 sediment samples were collected from streams and lakes of central and western USA during several independent investigations. All samples were dried at 40ºC and ground (< 100 μm) prior to scanning and laboratory analysis of up to 51 analytes. Calibration models developed by PLSR were tested by leave-one-out cross-validation. The MIR-PLSR models successfully predicted 35 of the 51 available analytes, based on the ratio of standard deviation to root mean square error of the cross-validation being greater than 1.5. For the major properties, PLSR models were successful (in order of accuracy) for Ti, K, Al, Fe, TOC, CEC, loss of ignition, P, Mg, Ca, silt, Mn and sand, and unsuccessful for Na, acid volatile sulphide and clay. Minor elements, Ga, Y, Cu, Li, Cs, V, Be, Bi, I, Rb, As, Sc, Cr, Ba, U, Zr, B, Sr, Co, In, Mo and Sn were also successfully predicted, but poorer predictions were obtained for Ge, Tl, Pd, Pb, Se, Ag, Cd, Br, Ni, Hg, Sb, Zn and Nb. Successful calibrations were dependent on the presence of analytes (e.g. Al, Ca, Fe, Mg, TOC) in known MIR absorbers e.g. minerals and organic matter, or due to their correlations (e.g. As, Cr, Cu, Ga, CEC, particle size) with MIR-active components. Unsuccessful models were due to a low number of diverse samples, low concentration, or absence of relationships with MIR absorbers. Thus, DRIFT-PLSR has the potential to simultaneously and rapidly (< 1 min) predict a range of properties of sediments, and might be adapted to field use with hand-held DRIFT instrumentation. The method therefore offers the opportunity to screen sediment samples for properties that are likely to affect toxicity of both inorganic and organic contaminants. Additional studies are in progress to better define the applicability of the method.

596

Non-extractable residues (NER) from xenobiotics in soil: a new classification and relevance in the risk assessment

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Anthropogenic organic chemicals are deliberately (e.g. pesticides) or unintentionally (e.g. polyaromatic hydrocarbons, chlorinated solvents, pharmaceuticals) released in major amounts to nearly all compartments of the environment. Soils and sediments as complex matrices provide a wide variety of binding sites and are the major sinks for these compounds. Xenobiotics entering these complex systems may undergo various turnover processes. They can be degraded chemically (e.g. photolysis), biologically by microorganisms, volatilised leached to the groundwater or taken up by living organisms or immobilised in the form of non-extractable residues (NER). NER formed during degradation of an organic contaminant in soil are commonly divided into two binding modes: *sequestered* (*type I*, adsorbed or entrapped) within the soil matrix or *chemically bound* (*type II*) via covalent bonds to SOM. However, recent studies with readily biodegradable compounds ($^{13}C_6$-2,4-D, $^{13}C_6$-ibuprofen, $^{13}C_3{}^{15}N$-glyphosate) showed that the NER identified by isotope mass balance are of biogenic origin. Therefore, the actual NER classification needs to be extended to a new classification scheme in terms of compounds forming the NER: into *xenobiotic NER*, which are composed of sequestered or bound parent xenobiotic and / or xenobiotic metabolites (*type I* and *II*) and into *biogenic NER* (*type III*, *bioNER*) containing natural biomass compounds. It is necessary to distinguish between the three types of NER in order to access properly the respective xenobiotic degradation rate and to estimate its potential risks for human and environment. For instance, biogenic NER which are composed of biomass compounds pose no risk for the environment. BioNER should be therefore clearly distinguished from the xenobiotic NER of type I and II, since the processes of abiotic NER and biotic NER

formations are competing processes. The relative importance of each of them will vary depending on environmental conditions, degradation kinetics, and sorption properties of the compound in question.

Mercury Biogeochemistry and Policy (II)

597

Mercury contamination in Lake Maggiore in the vicinity of a chlor-alkali plant: contamination of sediments and aquatic organisms

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A mercury-cell chlor-alkali plant in Lake Maggiore basin (North Italy) has severely affected the aquatic environment between the 1940s and the 1970s, with high levels of Hg contamination in sediments. The chlor-alkali plant is located on the river Toce, a tributary of the lake which flows into the Bay of Pallanza and drives contaminated sediments. Monitoring activities are carried out by the International Commission for the Protection of Italian-Swiss waters (www.CIPAIS.org), who collected data on contamination over a period of 15 years. Our objective was to investigate mercury contamination in the aquatic ecosystem of Lake Maggiore, with trend analysis of concentrations in sediments and biota in the last 15 years, in order to assess the potential risk for aquatic food chain and possibly for human health. THg concentrations were analyzed in lake sediment cores, in the bivalve *Dreissena polymorpha* and in fish specimens of *Coregonus lavaretus*, *Alosa fallax lacustris* and *Rutilus rutilus*. Samples were analyzed with AMA254 Automated Mercury Analyzer. Peak values up to 25 mg kg^{-1} d.w. were measured in a sediment core sampled in the Bay of Pallanza. Current Hg levels remain in the range of 0.5–1 mg kg^{-1} d.w. in the Bay of Pallanza and in the southern part of the lake. Values in *D. polymorpha* are higher in the Bay of Pallanza (up to 0,2 mg kg^{-1} d.w.) than along the N-S axis of the lake (up to 0,1 mg kg^{-1} d.w.) and concentrations have remained stable over the last 15 years. Values in fish are higher for *Alosa fallax lacustris* (0.096-0.266 mg Hg kg^{-1} w.w.) than for *Coregonus lavaretus* (0.070-0.111 mg Hg kg^{-1} w.w.) and *Rutilus rutilus* (0.059-0.147 mg Hg kg^{-1} w.w.). Concentrations in fish have remained stable in the last 12 years and are above the EQS of European Directive 2013/39/UE for Hg (0.02 mg Hg kg^{-1} w.w.), with potential risk for top predators of the food chains. Future monitoring programs will focus on understaning the persistence of Hg contamination in the biotic compartement. On this basis we conclude that the possible issues are: 1) remobilization of Hg from the river Toce during high flow events, 2) Hg inputs coming from a diffuse contamination of the whole watershed, 3) changes in methylation rates due to re-oligotrophication of the lake.

598

Retrospective monitoring of mercury in fish of European freshwaters

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For the implementation of the water framework directive (2000/60/EG) a European quality standard was defined with 20 µg/kg mercury (Hg) in fresh weight of aquatic organisms (EU-directives 2008/105/EG, 2013/39/EU). Although inorganic Hg and organic Hg (mainly monomethylmercury, $MeHg^+$) have to be assessed differently because of their different chemical behaviour and ecotoxicity - $MeHg^+$ is of special relevance concerning a secondary poisoning of predator organisms (organisms of a higher trophic level) -, only determination of the total mercury concentration in organisms is required. According to the literature the fraction of organic Hg in relation to the total Hg is for example in muscle of bream 95%, and 75 – 90% for eelpout, pike, perch, pikeperch and bream, respectively. Within a monitoring program to investigate the changes of HBCD concentrations in the environment a sampling campaign started in 2007 to collect samples of bream (*Abramis brama*) in different European rivers. Bream were collected in the period 2007-2012 from the rivers Tees/UK, Mersey/UK (no sampling 2009-2011), Götaälv/SE (no sampling 2009-2011), Western Scheldt/NL, and Rhône/FR as well as from Lake Belau/DE. The latter represents a site with little anthropogenic influence. The muscle tissue samples of 15 fish per site were homogenised and pooled. Samples of the homogenised bream muscle tissue from the different sampling sites as well as sole (*Solea solea)* muscle tissue from the sampling site Western Scheldt were analysed for their total mercury concentration by cold vapour- AAS (Direct Mercury Analyser) as well as for their monomethylmercury concentration by SID-GC/ICP-MS (species-specific isotope dilution - gas chromato-graphy - inductively coupled plasma-mass spectrometry). The results of this project should answer following questions: 1) Is the EQS in fish samples of European freshwater – as in bream samples of Germany (see data of the Environmental Specimen Bank operated by the German Environmental Agency; www.umweltprobenbank.de) – clearly exceeded for mercury? 2) How far are the concentration of mercury exceeded in fish of European freshwaters in contrast to samples of bream of the reference site Lake Belau (Germany)? 3) Are there any temporal trends in the concentration of inorganic and organic mercury? 4) Is the ratio of monmethymercury to the total Hg concentration at the different sampling sites and during the time constant?

599

Critical Review of Mercury Toxicity Reference Values (TRV) and Sediment Quality Values (SQV) for Protection of Fish and Benthic Invertebrates

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Mercury is a naturally occurring element that has been released from geologically stable forms into the environment through human activities. Mercury biomagnifies through the food web, and also has the potential for direct toxic effects on lower trophic levels, including benthic invertebrates. Risks posed to benthic invertebrates by chemicals in sediment are often initially screened by comparing chemical concentrations in sediment to sediment quality values (SQVs; also known as sediment quality guidelines or sediment quality benchmarks). SQVs are often developed as paired concentrations of chemicals in sediment, typically with a lower bound statistic that is believed to be associated with the absence of an effect (i.e., no effect) and an upper bound statistic that is believed to be associated with an adverse effect. During the presentation, we will present a recent reviews of mercury SQVs developed for characterizing mercury risks to benthic invertebrates (Conder et al., 2013). For comparison, data relevant to understanding causal relationships between mercury concentrations and sediment toxicity were also reviewed.

600

Identification of artisanal gold mining as a mercury pollution source in South Africa

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Mercury (Hg) as a hazardous metal has caused serious episodes of environmental pollution globally and disastrous human health distresses in the last decades. Mercury undergoes a series of transformations in the environment and is converted into inorganic and organic mercury. Methylmercury as the organic form, is listed high on the list of environmental pollutants, especially since it bioaccumulates through the food chain. In South Africa the magnitude of Hg pollution was relatively low on the agenda in 2000, although a study by Pacina *et al.* [1] has ranked the country as the second highest anthropogenic emitter. This data was largely based on the Hg emissions from coal combustion and artisanal gold mining, reporting that South Africa contributes more than 10% to the global Hg emission budget. South African Scientists have since then conducted further investigations and have reported that Hg emissions are significantly lower than previously reported [2]. In 2013, the UNEP Global Mercury Assessment Report [3] has identified artisanal gold mining as the latest source contributing the most to the global mercury budget. This presents South Africa with another challenge to fully understand its Hg footprint, as the area around Johannesburg was the main hub of artisanal gold mining activities from the 1880s until the 1910s [4]. The focus of this paper is to understand the role Hg has played in artisanal gold mining activities during the early stages of gold mining in South Africa. By quantifying the amount of Hg present in historical gold mining wastes, it is hoped that a possible prediction of Hg mobilisation, re-emission and persistence in the environment can be made [5]. Thus, more information is needed of the different stages in which Hg is applied, during amalgamation processes, to extract gold in order to evaluate rates of environmental Hg losses during the past mining operations. It is further hoped that by establishing emission factors, using data from previous and / or related studies, it will be able to estimate Hg loss rates from gold production rates. In doing so, it is also hoped that the Hg pollution footprint from artisanal gold mining in South Africa will add to better understanding the fate and transport of Hg pollution in the country's Water Resources. **Keywords**: Mercury Sources; Artisanal gold mining; Human health; South Africa; Water Resources.

601

Mercury Pollution, Population Declines and Species Loss: A Review of the Evidence

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Contaminants such as mercury and its effects on species have been relatively well studied. Recent research investigations have identified methylmercury exposure as an overlooked environmental stressor that can cause negative effects on wild animal populations, even in remote areas. Species sensitive to environmental degradation hold great potential as eco-indicator species. However, eco-indicators are often merely monitored over time without recognizing the processes and mechanisms related to the factors that reduce the overall health and performance of local populations. Mercury contamination in the United States is a well-documented "contaminant biology" example and continues to be an environmental and public-health issue of great concern for certain sectors of the

global human population. Documentation of the pervasiveness of this contaminant is a first step toward understanding the potential environmental health and ecological implications of mercury pollution, including species loss and potential population decline of biological organisms. Using weight of evidence approaches I synthesize and evaluate variation in mercury bioaccumulation and distribution across a broad gradient of physical, climatic, biotic, and ecosystem settings to identify the environmental conditions and ecosystem types and species that are most sensitive to mercury pollution. Implications of this work as it relates to the UNEP mercury convention will also be discussed.

602

Calculating mercury risk and evaluating remediation actions using Bayesian networks for the South River, Virginia, USA

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Ecological managers are required to implement one or more management options to reduce risk to ecosystem services without the direct integration of risk assessment and evaluation of management alternatives. Throughout the decision making process a manager must consider multiple physical, chemical and biological stressors on an endpoint as well as the interactions of these stressors and the resulting effects. In this study, we use Bayesian networks (BNs) in a relative risk assessment framework to assess risk to four biotic endpoints and four water quality endpoints and evaluate remedial options for the mercury contaminated site, South River, VA. Bayesian networks explicitly describe the effects of mercury toxicity on these endpoints, as well as the interaction between mercury and other stressors. Through communication with decision makers for the South River, we identified two management options for the site: bank stabilization and agricultural best management practices (BMPs). The management goals for the South River reach beyond simple mercury remediation. The primary goal expressed by managers is "no regrets." In other words, the managers do not want to make the site worse in any way, such as reducing mercury levels at the detriment of habitat, loss of other species or other environmental parameters. The BNs are able to represent the expected effects of a management option and potentially unintended consequences. We have integrated these remedial options into our BNs separately, as well as combined, for the biotic and water quality endpoints. We used the South River total maximum daily load (TMDL) modelling to estimate that 45-75% of nutrients and bacteria come from agricultural land. Agricultural BMPs either slightly reduce risk or do not change risk to endpoints. Even if total phosphorus or bacteria inputs are zero, there is little change in the risk distribution output because these parameters are not risk drivers for any endpoints. We are currently completing model parameterisation for bank stabilization. Our work provides the managers of the South River with a tool that describes how management options are expected to change mercury levels, as well as overall risk. As with many mercury contaminated sites, management for the South River is not a one-time decision. Our BNs can be updated with new monitoring data to inform future decisions for the site, which is important because the South River will be managed for another 70 years.

Toward sustainability: benchmarks, certification and LCA (II)

603

MEASURING THE ENVIRONMENTAL PERFORMANCE OF THE UK CHEMICAL INDUSTRY – A LIFE CYCLE APPROACH

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The UK chemical industry is one of the largest manufacturing sectors in the UK and is pivotal for many other industries. The sector is a major export source with a turnover of over £60 billion projected to grow by 16.3% in 2016. The chemical industry is characterised by large production volumes, complex technologies and vast distribution networks with environmental consequences such as natural resource depletion, emissions to air and water as well as waste generation. These environmental impacts are potentially significant but are currently unknown. \nLife cycle assessment (LCA) was used in this study to provide insight into the environmental impacts associated with this industry with emphasis on some of the key chemical sub-sectors. A "cradle to gate" approach was used to evaluate the environmental burdens of the sub-sectors. The methodology involved the identification of some of the key chemical sub-sectors and their individual component elements and an assessment of the average environment impacts of each of these sub-sectors. A subsequent estimation of the total environmental impacts of each chemical sub-sectors over five years was performed using production data sourced from the UK Office of National Statistics. The LCA data were sourced from Ecoinvent and GaBi 6® software was used for LCA modelling. The environmental impacts were calculated using the CML 2001 method. \nThe results give an insight into the hotspots along the supply chains and indicate opportunities for improvements. The use of LCA as a decision-support tool in the sector is also discussed.

604

Evaluation of ILCD Data Network Entry-level and PEF data quality requirements in LCA databases

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In its 2013 Communication on "Building the Single Market for Green Products", and in response to commitments in the Communication on "A resource-efficient Europe – Flagship initiative under the Europe 2020 Strategy", the Commission adopted a Recommendation on the use of the Environmental Footprint as methods to measure and communicate the environmental performance of products and of organisations. The ILCD Data Network (ILCD DN) on the data infrastructure that is critical for further uptake of these and other life cycle based methods for their successful implementation in business and policy decision support. It is a web-based infrastructure aimed at providing access to consistent and quality-assured life cycle inventory (LCI) datasets from various providers. To be part of the network, datasets have to fulfil ILCD DN entry-level quality requirements. Indeed, data quality requirements have been also developed for datasets to be used in studies that are in line with the PEF/OEF, many of which are common to PEF quality requirements, but others differ in the degree of strictness. This contribution describes the approach adopted for analysing the extent to which life cycle datasets available in LCA databases meet ILCD DN entry-level and PEF data quality requirements, together with key issues emerged from the analysis. The analysis has been carried out on a sample of datasets of ECOINVENT v3.0, GaBi and EIME databases and the findings have then been extrapolated to the database level and to other third-party databases.

605

Evaluation of the importance of impacts from potential deforestation for fiber-based products in the context of life cycle assessment for non-certified products

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Deforestation and land degradation is a major cause of environmental impacts, often associated with logging, mining, and crop or pasture land need. However, current inventory databases often do not contain information about potential deforestation in the background of wood-, fiber- and agro-based products. The goal of this study was to evaluate the importance of impacts from potential deforestation for fiber-based, and explore different ways to introduce this information in current life cycle inventory database. The impacts of 1 ha of deforestation were modelled starting from the ecoinvent process 'clear-cutting primary forest to arable land'. A specific process was modelled for deforestation associated with logging to account for carbon stored in the logs themselves. In order to evaluate the impacts of deforestation potentially associated with logging, we worked in collaboration with the Programme for the Endorsement of Forest Certification (PEFC). On a global scale, 13'000'000 ha are deforested per year and it can be assumed that 10% of deforestation relates to wood extraction. Therefore, there is approximately 1'300'000 ha deforested that is attributed to wood extraction. As a starting point, if overall deforestation associated with wood extraction is allocated equally among the entire wood extracted, one gets approximately 3.5 m2 of deforestation per m3 of wood extracted. Assuming that certified wood can be excluded from wood associated with deforestation, one gets an average of 4.66 m2 of deforestation per 1 m3 of non-certifiable wood. Such an analysis can be further refined, considering for example regionalization. As an example, using such allocation of deforestation, the carbon footprint from non-certifiable softwood is about 10x higher than from certified softwood and ecosystem quality impacts about 2.5x higher. For paper the differences are smaller due to the contribution of other activities. Using such allocation, paper coming from non-certifiable wood is between 5% and 25% more impacting than full certified paper for carbon footprint and ecosystem quality. These preliminary results show that it is important to evaluate whether deforestation may be present in the supply chain of fiber-based products, and if so, account for its impacts.

606

Defining and implementing a sustainability strategy in industry: LCA and C2C® certification

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Life-Cycle Thinking is usually identified as the central approach of manufacturing industries sustainable strategy, since the products are at the core of their activities. The implementation of Life Cycle Assessment (LCA) as a fact based practice for process and product environmental improvement is therefore essential. Many of the manufacturing industries using LCA as an eco-design driver and a strategic tool are

aware that LCA is not sufficient at its current level of development to address some specific issues for their products and processes, such as product life-cycle waste management, or specific toxicity risk assessment and limitation. Therefore, we observe a growing interest from industries for other Life-Cycle Thinking approaches, like Cradle to Cradle (C2C®), striving to propose an integrated perspective for industrial products and processes design. By using the case study of a linoleum floor covering from Tarkett producer, the aim of this presentation is to demonstrate existing complementarities between LCA and C2C® that could lead to extend the boundaries of industries' sustainability strategy by the development of consistent benchmarks (reference values or thresholds). Whereas LCA measures quantitative life cycle potential environmental impacts with the final aim of reducing them, C2C® provides qualitative technical guidance for improving materials and energy flows management along products' or processes' life cycle by maximising the benefits they provide to humans and ecosystems. The latter can be used as global design strategy guidelines and can also lead to products' certification. Therefore, based on C2C® certification criteria and considering the company's economic constraints, the C2C® approach can potentially lead decision makers to set both qualitative and quantitative benchmarks, such as restrictions on certain ingredients because of their toxicity, substitution of components or chemical compounds, redesign of the product assembly to improve product recyclability, development of end of life recovery chain, etc. A proposal on how LCA could bear C2C® shortages and vice versa is built from the identified limitations of both approaches. From a scientific point of view, the analysis and positioning of the two methodologies has proceeded thought the investigation of the different assessment directionalities (eco-efficiency vs. eco-effectiveness).

607

Is Carbon Footprinting an appropriate method to combine with Data Envelopment Analysis for environmental benchmarking? The CFP+DEA method

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Life-cycle (LC) approaches are currently used to provide sustainability benchmarks through combination with Data Envelopment Analysis (DEA), a linear programming methodology that measures the relative efficiency of multiple homogeneous entities (named decision-making units, DMUs). These joint applications, named LC+DEA methods, have already been applied to several production sectors, mainly agriculture and fishing. In particular, environmental benchmarks are of special relevance in the energy sector, as it is a key source of environmental impacts worldwide, e.g. in terms of climate change. Hence, we argue that the combination of Carbon Footprinting (CFP) with DEA may be an appropriate approach to evaluate and benchmark the performance of energy entities. A five-stage method is proposed to jointly implement CFP and DEA with the aim of providing benchmarks that can be of relevance for policy making in the energy sector. The stages of the method include (i) data collection for energy and material flows of each DMU, (ii) carbon footprint computation for each individual entity, (iii) calculation of operational efficiency and target operating points through DEA, (iv) CFP re-evaluation of inefficient units, incorporating the operational benchmarks obtained in the previous stage, and (v) interpretation of the results to facilitate decision making at political and company levels. Advantages of the CFP+DEA method include the consistent and quantitative minimisation of resources to attain efficient operational levels, while providing a useful mechanism to quantify and report environmental targets for industries and governments according to eco-efficiency criteria. On the contrary, the method presents certain constraints, some of which are common to those discussed in the available literature regarding the two tools (i.e., CFP and DEA) independently. It should also be noted that economic aspects are not thoroughly addressed through this method. Overall, the CFP+DEA method is presented as a robust approach to aid policy makers in the energy sector to avoid future potential bottlenecks, by tackling directly the issue of mitigating greenhouse gas emissions. The method profits from the advantages of the two independent methods, while avoiding some of their constraints thanks to their integrative application.

Developments in Environmental Quality Standards: bridging the gap between science and practical regulatory implementation (II)

608

Criteria for Evaluating and Reporting Ecotoxicity data (CRED) – Report from a ring test

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This presentation reports from the CRED-project, short for Criteria for Evaluating and Reporting Ecotoxicity data, aiming at strengthening the transparency, efficiency and robustness of risk and hazard assessments. This has been done by increasing the usability of peer-reviewed ecotoxicity studies in regulatory risk assessment of chemicals by providing guidance on how to report and evaluate ecotoxicity studies. Within the CRED-project we have performed a two-phased ring test with over 80 risk assessors from Europe, Asia and North America, representing academia, regulatory agencies, consultant firms and industry. In phase I the risk assessors were asked to evaluate ecotoxicity studies according the Klimisch method. In phase II the risk assessors was asked to evaluate the same ecotoxicity studies but now according to the newly developed CRED-evaluation method. The consistency of the reliability evaluation increased when using the CRED-evaluating method and the risk assessors evaluated the method to be more accurate, applicable, consistent and transparent in comparison to the Klimisch method. The results from the comparison were used to improve the CRED-evaluating method and to develop the CRED-reporting recommendations. The CRED-evaluating method include a set of reliability criteria as well as a set of relevance criteria, since both being equally important in the evaluating process of ecotoxicity studies for regulatory use. Both sets are accompanied by extra guidance material which includes examples of how the specific criterion should be used. The CRED-reporting recommendations are a guideline for peer-review publication of ecotoxicity studies. The guide instructs researchers how to produce studies with high reliability and high reproducibility, and thereby increasing the likelihood for inclusion in regulatory environmental hazard and risk assessments of chemicals. Moreover, a stringent reporting of studies allows editors, reviewers and regulators to evaluate the available information in a more structured and transparent way.

609

Deriving Environmental Quality Standards (EQS) for fungicides: the lack of biotests for aquatic fungi adds to uncertainty in EQS derivation.

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Classical aquatic risk assessment is based on toxicity to organisms representing a simple food chain from primary producers (e.g. algae) via primary consumers (e.g. waterflea) and secondary consumers (fish) to end consumers (birds, mammals). However, in creeks and small rivers the main energy input from primary production is not derived from algae and aquatic plants but from allochthonous material (e.g. leafs) that has been processed by detrivores like aquatic fungi. Hence, fungi play an important role for the functioning of those ecosystems. However, toxicity tests with aquatic fungi are scarce at best and data are missing for the majority of fungicides. This poses a problem for the derivation of environmental quality standards (EQS) for fungicides. Toxicity data for fungi seem to be mandatory for lowering the standard assessment factor (AF) from 100 to 10 when deriving a MAC-EQS (the EQS for short-term exposure). The EU TGD for deriving EQS states: "For substances with a specific mode of action the most sensitive taxa can be predicted with confidence. Where representatives of the most sensitive taxa are present in the acute dataset, an AF < 100 may again be justified". Fungicides have a specific mode of action and are designed for controlling fungi, thus these organisms may well be the most sensitive group and the lack of toxicity data for fungi represents a significant uncertainty in EQS derivation. It is apparent that toxicity tests and data for aquatic fungi are needed for a meaningful risk assessment for fungicides. This information gap was recently acknowledged in the new aquatic guidance document under the authorisation of plant protection products published by EFSA in July 2013. For the development of new bioassays it is essential to know the biodiversity of aquatic fungi as well as their functions in aquatic ecosystems. A second important need is answering the question which level of protection should be achieved. If they are treated the same as the currently considered taxonomic groups, aquatic fungi should be protected at the level of their structure (biodiversity) and their ecological function (ecological processes). EFSA however, proposes to protect aquatic fungi solely based on their function in ecosystems. Here we present a review of available information on the function and ecology of aquatic fungi. Consequences of setting different protection goals for biotesting and hazard assessment will be illustrated.

610

Deriving a Water Quality Standard for Iron from Field Evidence

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The derivation of a Environmental Quality Standard (EQS) for iron in UK freshwaters is complicated considerably by its behaviour in solution, which tends to result in precipitation. The use of total, rather than dissolved, iron as a measure of exposure concentrations in ecotoxicity tests provides a reasonable surrogate for the toxic component of solutions which have been aged for three hours. Laboratory studies in which adverse effects are observed due to precipitate formation may be of relevance to EQS derivation although they are not relevant for classification. Field data can be an important line of evidence in setting quality standards alongside conventional laboratory ecotoxicity data. Information from field monitoring is rarely used directly for EQS derivation, although it is used in some cases as supporting information, for example in the selection of the appropriate assessment factor used to derive the standard. There have been several analyses of field evidence aimed at deriving an ecologically acceptable concentration of iron reported in the literature, although these studies have not taken account of the effect of water chemistry on the ecotoxicity of iron. Recent studies on the ecotoxicity of iron to fish, invertebrates and algae have shown a clear effect of water chemistry on iron toxicity. As such, water chemistry should ideally be taken into account when establishing quality standards for iron. This study aims to take account of water chemistry conditions on the ecotoxicity of iron in assessing whether or not impacts are observed on ecological communities in real field conditions. Previous analyses of the effects of iron on aquatic communities have not taken account of the effect of water chemistry on the ecotoxicity of iron. Various sources of information indicate that some invertebrate families are amongst the most sensitive aquatic organisms to iron toxicity. The derivation of an EQS for iron in UK freshwaters follows a weight of evidence approach, and incorporates several distinct lines of evidence to draw conclusions about an ecologically acceptable level of iron exposure.

611

The minimal model community as a comprehensive ecological risk assessment framework

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There is a gap between the ecological modelling for risk assessment of chemicals and the risk assessment framework demanded for regulation as regards precision and ecological relevance of estimation, the quality and quantity of required data, and the generality of application. Most ecological models used in ecotoxicology need acute or chronic toxicity data for various endpoints and organisms. On the other hand, the testing framework at the screening level produces toxicity data from very limited test organisms (e.g. algae, daphnia and fish), and the conventional method of ecological risk assessment, e.g., the hazard quotient method, does not include any ecological factors (e.g. life history of species, interspecific interaction) which potentially affect the interpretation for the derived ecotoxicity data. Here I present a simple aquatic ecosystem model (Tri-trophic Ecological Risk Assessment Model: TERAM), which, consisting of three trophic levels, can interpret the ecological significance and evaluate ecological risks based on the restricted ecotoxicity data. The presented model includes essential ecological factors (interspecific interaction, age-and-stage structure, size dynamics, and life history scenario of growth and reproduction) and toxicokinetics-toxicodynamics elements, whereas it can work with the minimual three-species screening-level ecotoxicity data (the fish acute mortality, the daphnia immobility, and the algal growth inhibition). The benchmark of ecological risk in the presented method is the annual population growth rate of the top predator (the fish), which summarizes the direct effect to the fish population and the indirect effect through interspecific interaction. As case studies I conducted the community-level simulations for 4 agrochemicals (fenitrothion, pyridaphenthion, pretilachlor, butachlor), using ecotoxicity data from AQUIRE (US EPA). The stochastic simulations based on uncertainties of toxicity data and acute-chronic extrapolations revealed the risk induced by such uncertainties. TERAM was able to estimate ecological risks of chemicals that had contrasting toxicities between the different trophic levels (e.g. daphnia and algae) by a common standard (the population growth rate of the top predator), because TERAM took into account the indirect effect of toxicants which directly affected the lower trophic levels by converting the indirect effect through interspecific interaction into the population-level effect at the top predator.

612

Closing the gap between setting standards and taking regulatory decisions

T. Warn; P. Whitehouse, Environment Agency / Evidence

This paper builds on experience in using standards to take decisions. Such actions have involved costs of tens of billions of Euros. Some work on devising standards pays little attention to the processes and discipline by which standards must be used to decide action. This can lead to targets that are ineffective or too complicated, and this can promote bad decisions. Work on a new standard may yield a concentration that is declared "safe". If this is to be used as a serious standard, it needs to be coupled with a clear statement on how often the concentration can be exceeded. It should be the job of the creator of the standard to provide this. It this is not done, the responsibility is shunted on to the users of the standard.\n Standards should reflect the discplines that will apply to how they are to be used. A serious standard cannot be an absolute limit; it must be something like an annual mean or an annual percentile. This allows an unbiased estimate of compliance and classification, a correct process for deciding controls on potential polluters, and a capacity to know the true statistical uncertainties in all of these processes. A review of such calculations should be part of the process by which a new standard is devised. This will advise the extent to which it is worth going into details of chemistry or toxicology, and will help ensure that a new standard does its job.

613

Discussion

A. Peters, WCA Environment Ltd; P. Whitehouse, Environment Agency / Evidence

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (II)

614

Development and validation of a partial life-cycle test with Potamopyrgus antipodarum

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Molluscs, though the second largest clade next to the arthropods, have widely been neglected in environmental risk assessment schemes for chemicals, mainly due to the lack of standardised and broadly accepted test guidelines for molluscs. However, they are known to be uniquely sensitive to a number of endocrine disrupting chemicals (EDCs, e.g. organotins) and other substances (e.g. copper). Therefore the German Federal Environment Agency and the Department for Environment, Food and Rural Affairs of the United Kingdom supported the preparation of a *Detailed Review Paper* (DRP) *on Molluscs Life-cycle Toxicity Testing*[11].The DRP proposed *inter alia* the parthenogenetic mud snail *Potamopyrgus antipodarum* as standard test organism. In the *P. antipodarum* reproduction test the number of embryos in the brood pouch, reflecting the individual reproduction effort in snails, and adult mortality serve as main endpoints. The present study aims to develop and validate the partial life-cycle test on the reproduction of *P. antipodarum*. Here, results from two pre-validation studies of the reproduction test with the chemicals tributyltin (TBT) with nominal concentrations of 10 - 400 ng TBT-Sn/L and cadmium with concentrations of 3.13 - 25 μg/L at eleven laboratories are presented. The mean embryo number in snails exposed to TBT decreased with increasing concentrations. First results show comparable NOEC (65 to 160 ng TBT-Sn/L) and LOEC (160 to 400 ng TBT-Sn/L) values for TBT. The EC_{10} was between 109 (95%-CI: 44.6 - 268) and 132 ng TBT-Sn/L (95%-CI: 58.8 - 329) indicating a good inter-laboratory reproducibility with a coefficient of variation between 8.4% (EC_{10}) and 35.3% (LOEC). The good reproducibility is also reflected in the reproduction test with cadmium. EC_{50}-values varied between 5.55 (95%-CI: 3.14 - 8.35) and 19.9 μg/L (95%-CI: 16.4 - 24.0) with a coefficient of variation of 35.1%. The effect concentrations for TBT and cadmium are in good accordance with already published data. Both pre-validation studies show that the reproduction test with *P. antipodarum* is a well suited tool to assess effects of EDCs and other chemicals. [1] OECD. 2010. Detailed review paper on molluscs life-cycle toxicity testing. ENV/JM/MONO(2010)9, Paris, France. *Acknowledgement* - The authors thank the German Federal Envrionment Agency for funding (UBA projects 370861402 and 371165417).

615

Chronic exposure to two components of tritanTM copolyester on Daphnia magna and Moina macrocopa

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Tritan^{TM} copolyester, a novel plastic form manufactured by Eastman company, has been widely applied for packaging of beverages and food contact films as an alternative of bisphenol A. However, very limited information has been reported on the toxicity of tritan^{TM} copolyester among aquatic organisms. We investigated chronic toxicities of terephthalic acid (TPA) and 1,4-cyclohexanedimethanol (CHDM), two important co-monomers of tritan^{TM}, using freshwater crustacean including *Daphnia magna* and *Moina macrocopa*. Ten replicates with one neonate each were exposed to various concentrations of TPA (control, 0.1, 0.3, 1.1, 3.3, 10 mg/L) and CHDM (control, 1.2, 3.7, 11, 33, 100 mg/L). After 21 d of chronic exposure, the number of young per female and the number of young per brood of *D. magna* were significantly reduced as increased TPA concentrations. The number of young per female in *D. magna* exhibited slight decrease after exposure to CHDM.

In *M. macrocopa*, no significant change of survival and reproduction endpoints was observed by TPA and CHDM exposure. Since concentrations of TPA and CHDM that have been reported to occur in ambient waters are much less than the thresholds for effects on the endpoints studied here, direct impact due to tritan^{TM} exposure is not expected in ambient water. However, given the importance of reproduction, the consequences of endocrine disruption at environmentally relevant concentrations deserved further investigation.

616
Effects of benzophenone-3 exposure on endocrine disruption and reproduction in Japanese medaka (Oryzias latipes)
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Benzophenone-3 (BP-3) has been widely used in sunscreens and other cosmetic products to protect human skin and materials from the harmful effects of UV irradiation. While BP-3 has been frequently detected in surface waters, sediments and biota, only limited information is available on its *in vivo* toxicity, particularly on fish. In the present study the endocrine disrupting potentials of BP-3 and its underlying mechanisms were investigated. Adult Japanese medaka pairs (F0) were exposed to 4.7, 8.4, 26, or 90 μg/L of BP-3 for 28 days. Effects on reproduction, sex steroid hormones, and transcription of various associated genes were determined. Additionally, the adverse effects on F1 fish were further examined with subsequent exposure to 5.4, 12, or 30 μg/L of BP-3 for 30 d exposure after hatching. Chemical analysis of water confirmed transformation of BP-3 to BP-1, more potent estrogen agonist. After 14 days, plasma concentrations of testosterone (T) were significantly decreased in male fish. The 17β-estradiol (E2) to T ratio (E2/T) showed significant decreasing trends in both males and females. Overall down-regulation of steroidogenic genes in gonads such as *star*, *cyp11a*, *cyp17*, *hsd3b*, *hsd17b3*, and *cyp19a* was also observed. In the liver of the male fish, vitellogenin induction was significant following exposure to 90 μg/L of BP-3 at both transcriptional and protein level. After 28 days of exposure, the daily number of eggs produced per female was significantly reduced at 26 μg/L of BP-3. However, continuous BP-3 exposure of F1 eggs did not affect the hatchability. This study clearly showed that low level of BP-3 could alter endocrine balance or affect reproduction performance. Effects of longer term exposure of F1 fish warrant further studies.

617
Development of in-vitro tests for the assessment of reprotoxicity in fish
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There is now clear evidence that numerous xenobiotic compounds act as endocrine disrupters (EDs) in fish by affecting reproductive functions. Some of these compounds exert their action by binding to steroid receptors, and several well established in-vitro methods can assess this mode of action. However, few methods are available to detect non-genomic mechanisms of action. The developed in-vitro tests targeted specific enzymatic pathways that play a key physiological role in fish reproduction; such are testicular synthesis of oxyandrogens, ovarian synthesis of estradiol and maturation inducing hormones, and phase II metabolism of active steroids. This work proposes the use of gonad –different stages of sexual maturation- and liver subcellular fractions from different fish species, viz. carp –*Cyprinus carpio*– and sea bass –*Dicentrarchus labrax*– to carry out a first screening of compounds that act as EDs by inhibiting the synthesis and metabolism of active androgens and estrogens. A wide range of environmental pollutants, including pharmaceuticals, synthetic musks, organotin compounds, alkylphenols and polycyclic aromatic hydrocarbons (PAHs) have been investigated. Among the tested xenobiotics, the synthetic progestagens, drospirenone and norethindrone, had the strongest inhibitory effect on the synthesis of oxyandrogens (IC50s: 0.4-3.8 µM for CYP17 and CYP11b) followed by the PAH metabolite, 9-hydroxyphenanthrene (IC50s: 10-31 µM), nonylphenol, the polycyclic musks, galaxolide and tonalide, and the pharmaceuticals, fluvoxamine and fluoxetine. Interestingly, 9-hydroxyphenanthrene also inhibited ovarian P450 aromatase (CYP19) activity (IC50: 4.3 µM). Triphenyltin, tributyltin and nonylphenol inhibited the sulfation of estradiol (IC50s: 17, 18 and 41 µM) and the glucuronidation of testosterone and estradiol. The use of gonad subcellular fractions allow the detection of selected chemicals that by interfering with key enzymatic pathways might finally affect physiological processes, such as gamete growth and maturation in fish. These assays can be used to assess the effect of mixtures of individual compounds and environmental samples, and they may become useful tools for a more rational design of chemicals with reduced aquatic reprotoxicity.

618
A calibrated ecosystem model to assess the ecotoxicological risk of endocrine disrupters in aquatic environments
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Ecological risk assessment of chemicals entering aquatic environments primarily focuses on impacts to individual organisms, although the overarching goal of risk assessment is the protection of ecosystem services. Experimental approaches to characterize the ecological impact of chemicals are costly and time-consuming and thus, the consequences on whole ecosystems remain unclear. This is especially true for endocrine disrupters released to natural waterways through the effluent of wastewater treatment plants (WWTPs). This study takes up the challenge to develop an ecosystem model that can predict the effects of endocrine disrupters discharged in WWTP effluents on aquatic populations, as well as ecosystem responses occurring through ecological interactions. The data used to develop and calibrate the model come from a multi-year whole-ecosystem study performed at the Experimental Lakes Area (ON, Canada) where the synthetic hormone 17α-ethinylestradiol (EE2), a potent endocrine disrupter, was added to an experimental lake for 3 years at environmentally relevant concentrations. Physico-chemical and biological data were collected before, during and after EE2 additions. Endocrine disruption was observed in all fish species, including the collapse of fathead minnowafter the second year of EE2 additions. The ecosystem model that was developed and calibrated is an object-oriented model based on simplified AQUATOX equations for the species naturally present in the experimental lake (benthic invertebrates, phyto- and zooplankton, fish). For each fish species considered, two classes are used in the model: juveniles and adults. The model has been successfully calibrated with the experimental data obtained before EE2 addition and a sensitivity analysis revealed great consistency between the simulation results and current knowledge on such ecosystems. The developed model can simulate endocrine disruption based on (i) a decrease of gamete viability, (ii) a decrease of gamete production or (iii) an increase of fish mortality. The model results show that an increase in adult mortality of fathead minnows affected its population dynamics but also populations of other fish species. In addition, changes in direct and indirect competitive interactions can be an important ecosystem-level effect of endocrine disrupters and compensatory mechanisms are important in food-webs. The model is being used to understand and predict the risk associated with endocrine disrupters.

619
Assessment of endocrine disruption in Australian rivers using chemical, in vitro, in vivo and in situ techniques
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This study used an integrated approach combining chemical analysis of endocrine disrupting compounds (EDCs) and trace organic contaminants (TrOCs), multiple *in vitro* and *in vivo* bioassays, and *in situ* sampling to determine the level of endocrine activity in Australian rivers. A monitoring stage spanning 1-year, 73 sites and 285 water samples from various land-use scenarios (wastewater, industrial, residential, agricultural and undeveloped) provided data on the prevalence and concentrations of 57 unique chemicals. The endocrine disrupting potential was also quantified by utilizing 3 reproductive endpoints using the estrogen receptor (ER-), androgen receptor (AR-), and progesterone receptor (PR-) CALUX assays. Estrogenic activity was the main activity detected by *in vitro* assays, and was measurable in 19% of samples (>0.1 ng/L 17β-estradiol equivalents, EEQ). In many cases low concentrations of the synthetic estrogenic ethinylestradiol was a significant contributor to the estrogenic activity. *In vivo* lab experiments were then carried out using a native species (rainbowfish; *Melanotaenia fluviatilis*) and a prevalent exotic species (mosquitofish; *Gambusia holbrooki*) exposed to pure compounds (atrazine, bisphenol A, ethinylestradiol, estrone, propylparaban, pyrimethanil) at 10x environmental concentration as well as different mixtures (hormones and personal care products, industrial compounds, pesticides) at environmental concentrations. Vitellogenin protein and mRNA were quantified to measure the estrogenic response of exposed fish. Only the high concentration of estrone (750 ng/L) induced a significant increase in vitellogenin protein and mRNA expression. Exposure data generated using chemical and *in vitro* results along with effects data from the *in vivo* experiments were combined to generate a simple risk assessment. Mosquitofish were then sampled at 4 hot-spot locations and 2 undeveloped (reference) sites, as predicted by the risk assessment. Analysis of physiological and gonopodium morphology, histology, vitellogenin protein and body burden of priority chemicals are currently underway. This is the most comprehensive Australian study on endocrine disruption to date.

Sustainability of Swiss Chocolate Production

620
Ecosystem services, payment for ecosystem services, biodiversity: Securing future supplies and preserving biodiversity by paying cocoa farmers for Ecosystem Services
A. Felperlaan , CREM
Cocoa farmers that grow cocoa under the shade of indigenous trees, can make an important contribution to the preservation of biodiversity. This is optimal with a shade cover between 40% and 60%, thereby maintaining both biodiversity and decent production levels. In order to maintain this optimum, farmers must be trained in *best agroforestry practices* such as pruning, planting and soil fertility management. In addition to biodiversity conservation, ecosystem services (ES) from cocoa agroforestry include provision of a buffer for – or corridor between – nature reserves, natural control of pests, carbon storage and sequestration, food security and income diversification, water quality and quantity, and soil protection and soil quality. Some of these services are of direct local or regional benefit (improved soil fertility, clean and sufficient drinking water), others are more of global interest (biodiversity conservation and carbon sequestration). Up to now, cocoa farmers are not rewarded for their ES. This is a missed opportunity. Financial compensation for their services could possibly guarantee a better supply of cocoa and at the same time contribute to preserving biodiversity. One way of rewarding cocoa farmers for their delivered services could be to integrate Payments for Ecosystem Services (PES) as an optional (add-on) or independent module into existing certification schemes, the advantage being that it can benefit from existing infrastructures and a wide support from the supply chain. Alternatively, tradable 'biodiversity' credits could be developed (similar to carbon credits), however, this is very costly and time-consuming. Thirdly, a fund could be created for financing biodiversity friendly projects. Advantages of a fund include a more secure base for funding (also others than supply chain actors can donate) and the fact that it is inclusive to all farmers: it is open to certified and non-certified farmers as well as farmers that currently produce other crops. An important bottleneck of PES is to find buyers that are willing to pay for ES. In contrast to, for example, local drinking water companies that have a direct economic interest in clean and sufficient ground and surface water, external parties generally have no or little direct interest in ES. The value of ES can then be related to an improved corporate image or be part of the overall CSR strategy (for example, stop deforestation, increase productivity, improve farmer's income and preserve biodiversity).

621
Environmental impacts of chocolate in a life cycle perspective
N. Jungbluth, ESUservices Ltd

622
Overview of scientific evidence for chocolate health benefits
J. Van Wensem, TCB
The potential health benefits of consuming chocolate have only recently been discovered. Laboratory studies and observational and small scale experimental studies on humans have found that chocolate consumption not only lowers blood pressure, but that it may also have positive effects on serum cholesterol, platelet activity, endothelial function, and glucose tolerance. There are several bioactive compounds in chocolate that may promote alertness. Chocolate may affect stress levels by prompting serotonin production, which is a calming neurotransmitter. The number of publications concerning cocoa and chocolate is increasing steadily. More than 1,300 publications regarding "cocoa" or "chocolate" have been added to the PubMed database between 2005-2010; this was an increase of about 60% on the previous 5 years. Of such publications, an increasing proportion concerns the effects of polyphenols and, in particular, flavonoids on human biology and health. The biological mechanisms of flavonoids are still unknown. They have been related to, among other things, their antioxidant properties and to the fact that they increase the bioavailability of nitric oxide, which has vasodilatory and other beneficial effects on the cardiovascular system, but no scientific consensus exists. Studies that link chocolate consumption with health outcomes (instead of intermediate outcomes like blood pressure) are less common. There appears to be some scientific evidence to justify eating a moderate amount (approximately 2 oz) of dark chocolate daily.

623
Ecological-social-ethical interactions that comprise the life cycle along the way from growers to the end product
L. Kapustka, LK Consultancy

624
Economics and sustainability of Swiss Chocolate Production
P. Heid , Chocolats Halba

625
Discussion
J. Van Wensem, TCB

Bridging the gap between LCA scientific research and application by practitioners

626
How a collaboration works between industry & academia
V. Becaert, CIRAIG; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering

627
Issues related to recycling: consistency in system boundary and allocation approach reflecting reality
S. Zinck, Steelcase

628
Collaboration between industry and academia for more LCI data in the agrofood sector: the World Food LCA Database
N. Espinoza-Orias, Nestec Ltd / School of Chemical Engineering and Analytical Science

629
Making USEtox applicable by industry: the Toxtrain project
S. Humbert, Quantis

630
The need for harmonized water assessment methods for industry (including the WULCA work)
A. Prieur Vernat, GDF SUEZ / CRIGEN

631
Final discussion
S. Humbert, Quantis; U. Schenker, Nestle Research Center

Poster Abstracts

Ecotoxicology in tropical and polar regions (P)

MO001
Ecological risk assessment of agro-pesticides used in the Nha Trang area, Vietnam using the PRIMET model
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Vietnam with a population of 80 million is highly dependent on its agriculture and on its coastal marine resources. The country has seen a steadily increase of agro-chemicals to enhance the production of rice and other crops. Pesticide use has reached over 40 000 tons per year and is rapidly increasing. Only little research has been done to assess the risks that these pesticide pose to the Vietnamese people and to their environment. In this study pesticide data was gathered from 2007-2012 in the villages of Vinh Phuong, Vinh Ngok, Vin Than in the watershed of the Cai River, Nha Trang, Vietnam. Total surface area of the fields, pesticides in rice, vegetables, coffee and cashew and their application patterns were gathered along with hydrological data of water streams that drain the fields into the Cai River. Predicted environmental concentrations (PECs) of the pesticides and their risk to aquatic organisms was then calculated using the 1st Tier Ecological Risk Assessment PRIMET (a Decision Support System for assessing Pesticide RIsks in the tropics to Man, Environment and Trade). The PRIMET model is designed to yield a relatively worst-case risk assessment with interviewed data supplied from local farmers, pesticide characteristics, application scheme and physical senario of the assessed environment. PRIMET generated risk values, i.e Exposure Toxicity Ratio's (ETRs), the quotient between the environmental concentration (PEC) and the estimated safe concentration (PNEC) were calculated for all the pesticides in use and ecological risks were compared between crops and villages. Results are discussed in relation to ecological risks from pesticide applications to aquatic organisms of the local streams and the Cai River.

MO002
EVALUATION OF TOXIC AND GENOTOXIC EFFECTS IN SEDIMENTS OF COASTAL SYSTEMS LOCATED IN THE GULF OF MEXICO
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In our country studies on the occurrence and effect of pollutants in aquatic systems are scarce, despite the fact that the degree of pollution has increased on par with the development of human settlements and tourism, industrial, agricultural and oil activities. The aim of this study was to detect the presence of compounds with toxic and genotoxic effects in sediments of 5 coastal lagoons located in the Gulf of Mexico: 4 in Veracruz State (Pueblo Viejo, Tamiahua, Tampamachoco, Mandinga y Alvarado) and 1 in Tabasco State (El Yucateco) in order to delimit potential areas of risk and evaluate the effects of contaminants in species of economic importance. (*Crassostrea virginica y Mugil cephalus*). For the evaluation of the sediment toxicity, bioassays were performed with *Artemia franciscana* nauplii and for the detection of genotoxicity Chromotest microassay was applied and the degree of DNA damage in tissue samples *de A. franciscana, C. virginica,* and blood samples of *M. Cephalus,* was determined by the technique of single-cell electrophoresis (Comet Assay). The results show clear differences ($p < 0.01$) in the degree of deleterious effect on the DNA in the cells of organisms from each lagoon system, and the seasons examined. In the lagoons in the Gulf of Mexico, the degree of genetic damage was (High to low damage): El Yucateco >Tampamachoco > Mandinga > Alvarado > Pueblo Viejo > Tamiahua, being the rainy season where most deleterious effect was detected. The above results are consistent with pollutants levels recorded in the collecting sites. Also, with tests to assess the toxic and genotoxic effect of sediments have been detected potential risk areas in three locations in Pueblo Viejo Lagoon (mono verde, Barranco amarillo y Tamacuil), 3 in Tamiahua (La Loza, Cucharas y B. Corazones), 3 in Mandinga (Laguna Redonda, B. Kokina y B. Salazar), 4 in Yucateco lagoon (río Chicozapote, Boca río Zapote, Boca laguna y La Cuchupeta). In these places the implementation of activities such as cultivation of organisms, fishing or recreation may pose a risk to human health.

MO003
EVALUATION OF THE EFFECTS OF DETERGENT IN Lemna gibba L. Y Egeria densa Planch. MACROPHYTES
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Synthetic detergents are compounds used in large quantities in industrial and domestic activities. its formula is secret but generally contain surfactants (LAS, ABS) and additives that constitute persistent pollutants and are toxic to aquatic organisms. Because in our country there are few studies with detergents, and wastewater from Mexico city have a considerable contribution of these compounds, in this study an evaluation of the effects of detergents on biomass production, concentration chlorophyll, carotenoid production, levels of phenols and lipid peroxidation was performed in aquatic macrophytes: *Lemna gibba* and *Egeria densa*. Bioassays were carried out for 10 days in which the macrophytes were exposed to five concentrations of the surfactants, LAS and Triton X and 5 trademarks (Ariel, Ace, Foca, Rome and Salvo). In the results obtained significant differences between the responses of the control group and exposed to detergents were observed. The most toxic surfactant were: LAS and detergents containing enzymes (Salvo). A decrease in biomass and the level of chlorophyll and an increase in concentration of carotenoids, phenols and the degree of lipid peroxidation in all cases was observed. Because Mexico only 14% of the wastewater generated receive some type of treatment, it is important to conduct evaluations of sublethal effects of detergents in order to propose appropriate management measures to reduce the risk for the presence of these compounds in aquatic systems.

MO004
EVALUATING THE RESPONSE OF MICROALGAE Monoraphidium SP (CHLOROPHYTA) TO DETERGENTS
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The detergents are synthetic products used in large quantities for domestic and industrial cleaning, these compounds are formed by a surfactant and additives such as water softeners, preservatives, pigments, enzymes, foam stabilizers, dyes and perfumes. Since the studies performed to evaluate the effect of these compounds on aquatic organisms are scarce, the aim of this study was to determine the toxic effects of three surfactants LAS (lauryl alkyl sulphonate), lauryl dimethil (hidroxyetil) ammonium chloride and Triton X in the microalgae *Monoraphidium* sp. Bioassays were carried out with duration of 72 hours to assess the toxicity of detergents, determining the EC_{50} (effective concentration 50) which is the concentration that inhibits population growth by 50%, lipoperoxidation and pigments production. The results obtained a significant differences between the responses of the control group and algae exposed to detergents was observed. The toxicity of surfactants based in the calculated EC_{50} was (from greatest to least toxicity): Lauryl dimethil> LAS> Triton X. The decrease in chlorophyll levels was of 68%, 33% and 20% in the test with Hidroxyetil, LAS and Triton X, respectively was observed. The surfactant with greater oxidative effect was LAS. Because in Mexico only in 14% of the wastewater generated receive any treatment is important to continue conducting assessments of sublethal effects of detergents in order to propose appropriate management measures to reduce the risk by the presence of these compounds in aquatic systems

MO005
Assessing the ecological impact of banana farms on water quality using aquatic macroinvertebrate community composition
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In Costa Rica considerable effort goes to conservation and protection of biodiversity, while at the same time agricultural pesticide use is among the highest in the world. Several protected areas, some being wetlands or marine reserves, are situated downstream agricultural areas where large-scale banana farms constitute a major land use, with an average of 57 pesticide applications per year. The banana industry is increasingly aware of the need to reduce their negative environmental impact, but few ecological field studies have been made to evaluate the efficiency of proposed mitigation strategies. This study evaluated if benthic macroinvertebrate community structure is sensitive enough to detect environmental impact of banana farming, and thereby usable to assess improvements in management practices. Aquatic invertebrate samples were collected at 13 sites between March and April 2007, using kick-net sampling. Samples were taken both up- and downstream banana farms in fast flowing streams, with mostly cobbles for substrate in runs and riffles. The changes in community composition were visualized and tested on significance at the family level using ordination methods. Additionally, the

Biological Monitoring Working Party (BMWP) score system was applied along with a number of community composition descriptors. In total, 2890 specimens were collected, belonging to 14 orders and 49 families or taxa. The results indicates that surface waters immediately up- and downstream large-scale banana farms have different macroinvertebrate community compositions, with fewer sensitive taxa according to the BMWP-score values at the downstream sites. Rapid assessment using macroinvertebrate community composition thus appears to be a possible means to detect negative impact from chemical-intense agriculture. As the method is moderately time-consuming, low-cost and highly ecologically relevant it could become a useful complement to chemical analysis of pesticide residues in environmental risk assessment.

MO006
The application of the SPEARpesticides bioindicator in South Africa
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Chemical monitoring within aquatic ecosystems is often insufficient to determine quality as it does not take into account higher level effects on biota, instream speciation of chemicals, interactions with other physical impacts and variations due to longitude and time. To overcome this, instream biota are used as environmental indicators as they can integrate all of these higher level effects. However, many indices of biotic integrity are not sensitive enough to isolate community effects due to pesticide exposure as the communities also respond to other anthropogenic and natural stressors. A bioindicator system making use of macro-invertebrate traits, that is pesticide specific was therefore developed in Europe to overcome some of these challenges. The system, called SPEAR (SPEcies At Risk), has been applied as an indicator to link pesticide exposure and effects to insecticide toxicity. The aim was to apply the SPEAR system in South Africa to determine its applicability and effectiveness at linking pesticide exposure to changes in the macro-invertebrate community. For the purposes of this initial study, it was decided to use both the European and Australian $SPEAR_{pesticides}$ databases to determine if there are any differences between the results. This would in turn help to determine which database would be better suited for application within South Africa. The SPEAR systems indicated differing results when using the European and Australian databases with the Australian database indicating poorer ecological condition. These differences can possibly be attributed to the differing sensitivities between the two continents as studies have shown that there are differences between the sensitivities of tropical and temperate biota. The results indicated that all of the sites have either been exposed or not been exposed to pesticides; or that there was some interaction with other factors i.e. nutrients or salinity. It may also be that the Australian and European databases are not representative of the macro-invertebrate sensitivities to pesticides in this particular region. Further studies in different regions with more data points are needed to determine whether the SPEAR system is effective in South Africa.

MO007
Impact of the pesticide Vertimec® 18 EC on tropical freshwater plankton communities
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In recent years pesticides have been detected in many tropical freshwater ecosystems. However, little research has been done regarding their effects in surface water in tropical regions. In order to evaluate the effects of insecticide Vertimec® 18 EC over the tropical freshwater plankton communities we performed an outdoor mesocosm experiment with the presence of tadpoles species *Lithobates catesbeianus* (bullfrog). Six treatments were analyzed: two cases of no contamination (control and control + tadpoles) and two cases of pesticide contamination considered both with absence and presence of tadpoles: 0.002 $mL.L^{-1}$ (the concentration expected to reach the water body after application of the pesticide) and 0.008 $mL.L^{-1}$ (the LC_{50} for tadpoles of bullfrogs). Phytoplankton and zooplankton community and some environmental variables (pH, conductivity, dissolved oxygen, temperature, turbidity, chlorophyll *a* and nutrients) were monitored during 20 days. It was observed a total of 33 zooplankton species distributed between Rotifera (21), Cladocera (8) and Copepoda (4). Regarding phytoplankton, a total number of 94 taxa were identified. Chlorophyceae was the most diverse algae class with 43 taxa, followed by Cyanophyceae (18), Euglenophyceae (10), Bacillariophyceae (8), Zygnemaphyceae (6), Chlamydophyceae (4), Cryptophyceae (4) and Chrysophyceae (1). Treatments with the presence of the pesticide led to the mortality of all species of cladocerans and copepods. As an indirect effect of these and of the increase of nutrients, the density of phytoplankton tended to increase (decreased chlorophytes, whereas cyanophytes, cryptophytes and euglenophytes increased in abundance). In the treatment with 0.008 $mL.L^{-1}$ of the pesticide, all the tadpoles died 24 hours after the beginning of the experiment, suggesting that Vertimec® may be more harmful to tadpoles in natural conditions. As a result of the death of the tadpoles, the cladocerans and the copepods, there was an even more significant increase in the phytoplankton and rotiferans density. Therefore, due to the toxic mode of action of insecticides, cladocerans and copepods were more sensitive to Vertimec. Furthermore, indirect effects also occurred to phytoplankton and to rotifers. These results highlight the importance of experiments in semi-natural systems for assessing the direct and indirect effects of contaminants on water ecosystems.

MO008
Macromelanophage Centers (MMCs) and PAHs in Ariopsis felis from the southern Gulf of Mexico
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Five individual fish (*Ariopsis felis*) were collected in each of 16 sampling stations in the southern Gulf of Mexico, on the coast of the Mexican states of Tabasco and Veracruz in June, 2011. Fish were dissected, livers and bile extracted, and frozen in liquid nitrogen until analysis. A portion of the liver, the kidney and spleen were preserved for histology. Macromelanophage centers (MMCs) in liver, kidney and spleen were assessed by number and area. 10 sections of each organ and fish were analyzed microscopically using image analysis software (ImageJ). Fluorescent aromatic compounds (FACs) were measured in bile by fixed-wavelength fluorescence, and the concentrations of hydrocarbons (Total PAHs, low molecular weight [LMW-PAHs] and high molecular weight [HMW-PAHs] PAHs) were measured in liver by gas chromatography. Prevalence of MMCs was higher in the spleen (70% of all fish), then in the liver (42.5%), and the lowest in the kidney (16%). Total PAHs in the liver were in the range from 324.3 to 4,980.8 µg/g; Low molecular weight PAHs were in the range from 236.1 to 1632.8 µg/g, and high molecular weight PAHs in the range 68.8 to 4422.0 µg/g. In the case of fluorescent aromatic compounds, the concentration ranges were, for Benzo(a)Pyrene from 0.07 to 0.47 µg/mL; Pyrene was found from 0.13 to 0.66 µg/mL; Nafthalene from 13.2 to 53.6 µg/mL, and Fenanthrene from 16.1 to 95.4 µg/mL. The concentrations of low molecular PAHs in the liver, and the metabolites of PAHs in bile (FACs) were higher than those of high molecular weigth., indicating a petroleum source for this hydrocarbons. This is confirmed by the higher concentrations of methyl-naphthalenes compared to naphthalene. There was no relation between MMCs and PAHs, but there is a relation with some organochlorine pesticides. The concentrations of PAHs in the liver, PAHs metabolites and MMCs were higher in the catfish in this work, than the fish from the same species in the oil producing zone in the southern Gulf of Mexico. This work gives useful information to understand biomarker responses in relation to pollutant concentrations for these two species in the Gulf of Mexico, and their use in environmental monitoring and assessment.

MO009
Pesticide contamination of surface water in Guadeloupe and Martinique (French West Indies): co-occurrence of compounds related to past and current agricultural uses
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In the French Lesser Antilles (Guadeloupe and Martinique), in application of the Water Framework Directive, local water agencies have implemented chemical monitoring in a large number of rivers. A total of 25 and 28 sites in Guadeloupe and Martinique, respectively, are sampled every month in order to follow water concentration of more than 40 molecules identified as "priority substances"; "priority hazardous substances" and "hazardous substances". Both historical and insular tropical contexts confer to Guadeloupe and Martiniquespecificities in regard to the nature of the pollutants that are found in freshwaters. In the past, many organochlorines, in particular chlordecone and technical HCH, were used in bananas' plantations. Nowadays, pesticides, such as glyphosate, AMPA and asulam, used as herbicides, and azoxystrobin, imazalil or thiabendazol, used as fungicides, can be found in freshwaters of Martinique and Guadeloupe. The aim of this study was to assess water chemical quality in these two islands considering both the European Regulatory requirements and local specificities in terms of pesticide uses. Data collected from 2008-2012 and 2009-2011 inMartinique and Guadeloupe, respectively, were provided by the local water agencies. Annual average concentrations of pollutants most commonly found in freshwater were calculated and analyzed using Principal Component Analysis. The results showed that organochlorines are the most frequently detected pesticides in surface waters of Martinique and Guadeloupe. These compounds were found mainly in rivers of the North Atlantic Coast of Martinique and the South of Basse-Terre in Guadeloupe. In these areas, chlordecone was found at the highest concentrations (*e.g.* 2.73 µg/L in theRougeRiver, Martinique in 2011; 2.9 µg/L downstream of theGrandeAnseRiver inGuadeloupe in 2010). In the same areas, high concentrations in fungicides were found in some sites (e.g. 1.72 µg/L thiabendazole in The Pocquet River,Martinique, in 2012). Moreover, in the central and southern parts ofMartinique, multi-contamination has been observed which includes organochlorines (at lower concentrations than in the North), glyphosate, AMPA, monuron, and diuron. In addition to pesticides, hydrocarbons (e.g. benzo[a]pyrene and fluoranthrene) were present. In Guadeloupe, these compounds were also detected but at lower

concentrations than in Martinique.

MO010
Pollutants and Biomarkers in Fish from the Southern Gulf of Mexico
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Five individual fish were collected in each of 86 sampling stations in the southern Gulf of Mexico in June, 2012. The fish were of two species: a catfish *Ariopsis felis* and a flatfish *Scyacium gunteri*. Fish were dissected, livers and bile extracted, and frozen in liquid nitrogen until analysis. A portion of the liver, the kidney and gills were preserved for histology. The expression of five genes (Citochrome P-450-1A, Glutathion-*s*-Transferase, catalase, methallothionein and vitellogenin) was measured in liver. Hyperthophia in gills, granuloma in liver and kidney, and macromelanophage centers (MMCs) in liver were assessed. Fluorescent aromatic compounds (FACs) were measured in bile, and the concentrations of six metals (Cr, Cd, Pb, Hg, Ni and V) and hydrocarbons (low molecular weight [LMW-PAHs] and high molecular weight [HMW-PAHs] PAHs and total hydrocarbons) were measured in liver. Additionally, fish length and weight we recorded. Three research questions were investigated: 1) Are there differences in biomarker responses in the two fish species?; 2) Do fish size and weight affect biomarker response? If so, use as covariates; and 3) Is there a relationship between pollutant concentrations and biomarkers after removing the effect of covariates? Multivariate methods were used to answer the questions, RDA for gene expression and CCA for histology. There were significant differences in gene expression for the two species, with catfish having higher expressions, and for histology, with flatfish having more lessions. Weight was related to both gene expression and lesions, and was used as covariate. Only metals were related with gene expression (6.1% variance, and weight 0.6%) in flatfish, and with FACs in catfish (27% variance, and 0.2% for weight). There was no relationship between pollutants and histological lesions in flatfish, and with hydrocarbons (22.2% variance) in catfish. Particularly there was a strong relationship between HMW-PAHs and granuloma in the kidneys. This work gives useful information to understand biomarker responses in relation to pollutant concentrations for these two species in the Gulf of Mexico, and their use in environmental monitoring and assessment.

MO011
Comparison of Vertimec ® 18 EC effects to Ceridaphnia silvestrii for culture contaminated water and samples of contaminated artificial mesocosms.
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Vertimec ® 18 EC is a powerful pesticide that even at low concentrations causes toxicity to aquatic organisms. *In situ* uncontrolled factors can influence the contaminant effect, differentiating the commercial product toxicity from contaminated samples *in situ* toxicity. This study evaluated and compared the direct effects of Vertimec ® 18 EC for the cladoceran *Ceriodaphnia silvestrii* using contaminated mesocosms samples and culture contaminated water with the pesticide. The mesocosms was divided in three cases of contamination: without contamination (control), 0.002 (case 1) and 0.008 mL L^{-1} (case 2) and this concentrations correspond to the pesticide concentration in runoff water of strawberry crops and EC_{50} for tadpoles *Lithobates catesbeianus* respectively. Each case was carried out with (a) and without (b) tadpoles presence, used to simulated complex biological relationships. Samples were taken in 3, 96 and 192 hours after contamination in addition the sample before contamination. Nutrients, chlorophyll *a*, pH, dissolved oxygen, temperature and conductivity were monitored during the experiment. As the concentrations used in the *in situ* experiment are highly toxic to cladocerans, in order to find the EC_{50}, 48h, the mesocosms samples were diluted in 6 concentrations (100%, 50%, 25%, 12.5%, 6, 25% and 3.12%). For the toxicity tests with pesticide application in the culture water, the concentrations used were 62.5, 125, 250, 500 and 1000 nL L^{-1}. For the first mesocosms samples (3h) the EC_{50}, 48h, were 18.41% (368.2 nL L^{-1}, case 1a) and 10.38% (207.6 nL L^{-1}, case 1b) and less than 3.12% (62.5 nL L^{-1}, 2a and 2b). In both cases 1a and 1b toxicity values decreased over time, being 29.73% (594.6 nL L^{-1}) and 46.65% (933 nL L^{-1}) in samples of 192h, respectively. However, to cases 2a and 2b, the toxicity values remained lower than 3.12% (62.5 nL L^{-1}). The control without tadpoles showed no toxicity, whereas with tadpoles showed toxicity after 192 hours due the increased ammonia concentration excreted by tadpoles, considered toxic to cladocerans. The EC_{50}, 48h, for culture contaminated water was 81,64 nL L^{-1}. These results indicate high toxicity of Vertimec ® 18 CE to *C. silvestrii*, and highlighted the importance of use ecosystem models to understand the real effects of pesticides, since the toxicity values for mesocosms samples were lower than in culture contaminated water. \n

MO012
Metal pollution in eutrophic environments. Is the toxic effect of cadmium on Danio rerio embryos modified by the presence of cyanotoxins?
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Aquatic environments are exposed to different hazards that affect their biota. Eutrophication is observed in lakes and ponds enriched with nitrogen and phosphorus from agricultural fertilizer discharges and secondary wastewater treatment plant effluents; blooms of noxious, toxin-producer cyanobacteria are frequently observed in these conditions. On the other hand, the discharge of toxic metals and pesticides adds stress conditions to hydrobionts. Usually, aquatic organisms in polluted environments are exposed to multiple and different types of stressors, and it is important to determine the combined effects produced by them. In this study, the single and joint effect of cadmium (Cd) and aqueous crude extracts (ACE) of one toxigenic strain of *Microcystis sp.* (VU-5) were evaluated in early life stages of *Danio rerio*; the methodology used was that indicated in the OECD guideline 236 (Fish Embryo Acute Toxicity Test). *Microcystis* biomass was grown under laboratory conditions in autoclaved Z-8 medium, and microcystin content was determined with a commercial ELISA kit; a controlled stock of *D. rerio* reproducers were used to obtain fertilized eggs. Embryos of 2 hours post fertilization (hpf) were exposed to selected Cd and *Microcystis* extract concentrations during 96 h; in addition to the effects indicated in the guideline (coagulated embryos, no somite formation, tail not detached, and no heart beat), teratogenic effects and hatching percentage were recorded. Once the acute effects were determined, 2 hpf embryos were exposed to the LC_{50} value of the *Microcystis* ACE in combination with the same Cd concentrations tested previously (2.5, 5, 10, 20, and 40 mgL^{-1}). LC_{50} were 335 mg L^{-1} and 12.33 mg L^{-1} for Cd and ACE, respectively. The LC_{50} for the Cd+ACE mixture was significantly lower (2.85 mg L^{-1}) than for the single exposure to Cd, indicating increased toxicity when both stressors act jointly. Reduction in pigmentation, delay in development, and teratogenic effects (edema, body deformities, and no eye formation) were mainly observed in embryos exposed to *Microcystis* extracts, and increased in frequency with the Cd+ACE mixture. The toxic effect of Cd increased when microcystins acted at the same time; teratogenic effects of ACE exposure were also documented. It is important to take into account the effects of in situ produced stressors (e. g. cyanotoxins), because they can increase the toxic response of aquatic biota to chemical pollutants.

MO013
Brain cholinesterase response in the climbing perch fish (Anabas testudineus) after exposed to Vitashield 40EC.
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Abstract High use of pesticides in intensive rice farming in the Mekong Delta in Vietnam pose a hazard to the environment and people's health. Chlorpyrifos ethyl is a commonly used insecticide, but little research on the negative impacts of this organophosphate exists on the aquatic environment on tropical conditions. Both acute and sub-acute toxicity tests were carried out in a static and non-renewable system to investigate the effects of chlorpyrifos ethyl on brain cholinesterase (ChE) of native climbing perch fingerling (*Anabas testudineus*). Environmental parameters, such as dissolved oxygen, water temperature, and pH, fluctuated similarly to field conditions. In LC_{50}- 96h test, fingerlings of climbing perch were randomly exposed to five levels of chlorpyrifos, from 0.8, to 4.5 ppm and one control treatment with three replicates. Five levels of chlorpyrifos, 1, 5, 10, 15 and 20% of the LC_{50}- 96 h value, were tested to assess the sensitivity and recovery of brain cholinesterase activity in climbing perch fingerling exposed to chlorpyrifos. The results showed that chlorpyrifos was highly toxic to climbing perch, with a 96-h median lethal concentration (LC_{50}) of 1.73 ppm**.** Chlorpyrifos also caused long-term ChE inhibition, with 30% inhibition remaining after 96h forthe four highest concentrations. Recovery of brain ChE activity was very slow, and at termination of the experiment after seven days, the recovery of brain ChE activity was still significant lower in fish from the four highest concentrations as compared to the control. The finding form this study suggest that insecticides, such as organophosphates, can have long lasting sub-lethal effects on aquatic species in the Mekong Delta. Keywords: *Anabas testudineus, Chlorpyrifos ethyl, Acetylcholinesterase, pesticide impact, rice farming, Mekong Delta*

MO014
Pesticide run-off during rain events in Caribbean lowland agricultural area of Costa Rica
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The Caribbean lowland of Costa Rica is characterized by intensive agricultural activities like banana and pineapple plantations but also by its coastal canal and lagoon system with a rich biodiversity and important ecological value. Agrochemical use is normally high and can lead to the pollution of the aquatic environment by run-off following the heavy rains. To evaluate the potential environmental impact of pesticides in the lagoon system the understanding of the factors that contribute to pesticide runoff in the tropics is important. In this study pesticide runoff was followed in a stream that receives water from a banana and a pineapple plantation during rain events and a simple run-off model was applied to estimate the amount of the pesticides contained in the surface runoff (1). Water samples were collected before, during and at the end of rain events in the stream 500 m from the banana and pineapple plantation with an estimating drainage area of 260 and 400 ha respectively. Pesticides commonly used in these agricultural activities were analyzed in the water samples. Discharge of the stream was measured during the sampling and compared with precipitation data. The herbicides ametryn, diuron and hexazinone, commonly used in pineapple, were detected most frequently, with the highest concentrations found for hexazinone of up to 35 μg/L. Other frequently found pesticides were the insecticides carbaryl, chlorpyrifos, diazinon and ethoprofos with a maximum of 2.3 μg/L. The fungicides chlorothalonil, epoxiconazole, metalaxil and triadimenol were detected less frequently, in concentrations of up to 1.3 μg/L. During a rain event of more than 100 mm the highest runoff was found for the herbicides with a total estimated runoff of almost 15 kg/day and for the insecticides and fungicides, for which loads of 0.56 and 0.79 kg/day were estimated respectively. With the model the highest runoff potential can be expected for the pesticide applications in the pineapple plantation, characterized by higher field slopes, lack of buffer zones and the high use of herbicides which seems consistent with the observed loads found in the stream. This study discusses how the run-off model can be used to derive maximum predicted environmental concentrations (PEC) of pesticides and to assess the environmental risk of these pesticides on native aquatic organisms and the implementation for risk reduction strategies. 1) Berenzen N, et al. Chemosphere. 2005,58, 683-691.

MO015
Assessing exposure risk of agrochemicals on tropical aquatic species using Species Sensitivity Distributions (SSDs) in Nha Trang, Vietnam
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Abstract Run-off transport of pesticides from agricultural fields to adjacent land and watersheds is posing a growing threat to non-target species. The aim of this study was to assess the toxicity of two pesticides, the insecticide Furadan (Carbofuran) and the organophosphate herbicide Glyphosate on native Vietnamese fish, arthropods, coral and algae species in the Nha Trang area. The species sensitivity distribution method (SSD) was used to compare relative sensitivities to the pesticides. Since most ecotoxicological studies of pesticides have been carried out with freshwater species in temperate regions, geographical differences in sensitivity and the possibility of extrapolating temperate toxicity data on tropical species were evaluated. LC_{50}/EC_{50}-96h and 7 day standard toxicity tests were analyzed for mortality and immobility for vertebrates and growth inhibition for invertebrates. Additional aquatic single-species LC50 or EC50 values fromfish, crustaceans and algae were collected from the open literature and existing toxicity databases e.g. US Environmental Protection Agency (US-EPA) ECOTOX database www.epa.gov/ecotox. The E_TX 2.0 Program was used for estimating HC5 and HC50 values, fitting a log-normal model to the data assessed by Anderson-Darling goodness-of-fit test at $\alpha = 0.05$. Carbofuran was more toxic to crustaceans and fish than to the herbicide Glyphosate (96h L(E)C50 (μg/L); fish *Lates calcarifer* = 139, shrimp *Litopenaeus vannamei* = 78, lobster *Panulirus homarus* = 13,9) whereas Glyphosate was more toxic to the algal species (96h L(E)C50 (μg/L); *Chaetoceros muelleri* = 10652, *Skeletonema costatum* = 29837 and *Acropora formosa* = 6000 (NOEC value). When comparing tropical and temperate fish exposed to Carbofuran and Glyphosate, results indicate no statistically significant difference in species sensitivity. Example from fish (*L. calcarifer*); T-test assuming equal variance, t = -0.3, p = 0.8 (Carbofuran) and t = 0.7, p = 0.5 (Glyphosate). Results show no statistical differences between tropical and temperate regions (fish and crustaceans data) suggesting that the SSD method can be used for ecological risk management in tropical regions.

MO016
Effect of pesticides used in banana and pineapple plantations on aquatic ecosystems in Costa Rica
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Current knowledge on fate and effects of agricultural pesticides comes mainly from temperate ecosystems. More studies are needed in tropical systems in order to assess contamination risks to non-target endemic tropical species from the extensive use of pesticides e.g. in banana and pineapple plantations. In this study, acute laboratory toxicity tests with the organophosphate pesticides ethoprophos and chlorpyrifos were conducted on two Costa Rican species, the cladoceran *Daphnia ambigua* and the fish *Parachromis dovii*. Tests showed that chlorpyrifos was more toxic than ethoprophos to *D. ambigua* and *P. dovii* and that *D. ambigua* was also more sensitive than P. dovii to both pesticides. Additionally, bioassays were performed by exposing *Daphnia magna* and *P. dovii* to contaminated water collected from the field. Chemical analyses of field water revealed that fungicides were generally the most frequent pesticide group found, followed by insecticides/nematicides and herbicides. The bioassays and values obtained from the literature confirmed that *D. magna* was more sensitive to pesticide contamination than *P. dovii* and that *D. ambigua* was more sensitive than *D. magna*, suggesting that the native cladoceran is a more suitable test species than its temperate counterpart. Species sensitivity distributions for both fish and arthropods showed no significant difference in sensitivity between tropical and temperate fish and between the tropical and temperate species exposed to chlorpyrifos in this study. Cholinesterase activity (ChE) was also measured in *P. dovii* in laboratory tests in order to assess the applicability of this biomarker. ChE inhibition in *P. dovii* was observed in the lab at levels below the LC_{10} of both ethoprophos and chlorpyrifos, confirming that ChE is an efficient biomarker of exposure. Both indigenous Costa Rican species used in this study were found to be suitable standard tropical test species. Further studies are needed to investigate how protective the safe environmental concentrations, derived from LC_{50} of native tropical species, are for protecting tropical aquatic natural communities.

MO017
Insect diversity on high-input, low-input and organic banana farms
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High intensity of pesticide use in banana production is problematic not only for human health and the surrounding environment, but can threaten the provision of ecosystem services on which farm productivity depends. This research investigates the effects of varying pesticide-use intensities on on- farm insect diversity, using three different types of farm management systems: high pesticide input conventional system, reduced pesticide input conventional system and organic system. Insect sampling was done using pitfall and yellow bowl traps, left for a 24-hour period at 2 locations inside the banana farm, at the edge of the farm, and in adjacent forest. Species were classified to family level and then morphospecies. Insect species community composition and diversity were compared using multivariate statistics with ordination analysis and Monte Carlo permutation testing, and revealed that each of the management systems were significantly different from each other for both trap types. Insect diversity decreased as production management increased its pesticide use. Reduced insect diversity resulted in fewer functional groups and fewer insect families assuming different functions essential to ecosystem health. Organic farms had similar species composition on the farm compared to adjacent forest sites, whereas species composition increasingly differed between farm and forest sites as pesticide-use intensity increased. We conclude that while organic production has minimal impact on insect biodiversity, even small reductions in pesticide-use intensity can have a significantly positive impact on on-farm insect biodiversity and functional roles supported.

MO018
Feasibility of different colored clays for the composition of formulated sediments regarding pH, dissolved oxygen and metal availability parameters for cultivation of Hyalella azteca
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The clay fraction plays an important role in the sediment compartment of water bodies due to its high capacity of reaction and adsorption. This work evaluates the implementation of several colored smectite clays obtained commercially for the composition of formulated sediments. Stabilization capability of pH and dissolved oxygen under mild aeration and presence of organic matter (*Elodea* sp.), measured on a daily basis for 15 days, as well as the bioavailability of metals in the water

column (Al, Fe, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn) were analyzed. The analysis of metals in water was done with both surface and interstitial fraction. The methodology of Silverio (1999) was used to evaluate bioavailable metal concetration, and total metal concentration by acid digestion method suggested by EPA (1996). The metal content was determined by ICP-OES analysis. Results showed that dissolved oxygen remained above 4 mg L^{-1} throughout the experiment for all tested clays, in agreement with the minimum recommended value for cultivation of *H. azteca*. The values of pH became progressively more alcaline along the experiment for all the clays. The sample containing green clay had more stable responses and closer to the recommended value for the cultivation of epibenthonic organisms (between 7.2 and 7.7). There was greater availability of free metals in intersticial water than in the water column, which may cause greater toxicity for bentonic organisms compared to creatures present in the water column. The clays used in the present work showed high level of said metals as result of impurities responsible for its colors. Process FAPESP 2012/14583-5

MO019

Effects of Triclosan on the transcription expression of CYP1A, CYP3A and their enzymes activity in the Yellow Catfish (Pelteobagrus fulvidraco)

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Triclosan (5-chloro-2-(2, 4-dichlorophenoxy) phenol, TCS) is widely used in personal care products, industrial, veterinary and household products as a spectrum antibacterial agent. TCS can be universally detected in aquatic ecosystem due to its partial removation by the wastewater treatment process. However, limited information is provided about its potential adverse impacts on aquatic organisms, especially upon the expression of genes related to detoxification system and their enzymes activity of fish. We first cloned CYP1A, CYP3A and Alpha-GST of Yellow catfish (Pelteobagrus fulvidraco), an indigenous fish species in Pearl River Delta, South China. The gene expression of CYP1A and CYP3A and GST and their changes of enzymes activity of Phase I (EROD, APND and ERND), Phase II enzymes (GST, CAT) and MDA in the liver of Yellow catfish exposed to TCS (0.5, 5, 50 and 500 μgL-1 TCS, respectively) for 24h, 72h and 168h were determined by Real-PCR and a spectrofluorometer approach. The results showed that CYP1A mRNA expressions was significantly induced after TCS exposure for 24h and then significantly inhibited after 72h and 168h.Expressions of CYP3A were significantly inhibited for 24h and 72h but significantly induced for 168h at relatively low concentrations(0.5 and 5 μgL-1 TCS) while CYP3A was always induced at the highest concentration (500 μgL-1 TCS). GST mRNA expressions were significantly induced after 24h, slightly inhibited for 72h and remarkablely induced at the lowest concentration(0.5 μgL-1 TCS) while inhibited at the highest concentration. EROD, GST and MDA activities were significantly inhibited at 24h. ERND and EROD activities were significantly induced at the lowest concentration and inhibited at the higher concentration for 72h. Similar trends were observed for GST activity at 72h. CAT and MDA activities were induced at 72h while slightly inhibited at 168h at low concentrations. In general, ERND, EROD, CAT and MDA activities displayed stronger induction at the lower concentration (0.5μgL-1) than the ones at the higher concentrations at 72h, while APND, ERND, CAT and MDA activities showed more significant change at the concentration of 50μgL-1 at 168h. All enzymes tested in this study exhibited dose-effect relationships to some extent. EROD and GST were more sensitive in response to TCS exposure compared to other enzymes.

MO020

Erythrocyte oxidative damage and antioxidant defense system of traíra, Hoplias malabaricus, injected with crude extract of cyanobacteria, Radiocystis fernandoi.

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The erythrocytes have vital functions in the oxygen and carbon dioxide transport and hydrogen ion buffering. The microcystins (MCs) are toxins produced by cyanobacteria that may increase the free radicals and induce oxidative stress. In this context, this study evaluated the toxicity of MCs present in the crude extract of *Radiocystis fernandoi* on the blood cells of traíra, *Hoplias malabaricus*. Fish were separated in control group (VC-injection with saline 0.9%) and two groups injected intraperitonial with MCs (100 μg g^{-1} body mass): MC12h and MC96h. Twelve and ninety-six hours after injection the blood was sampled and the activity of superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione peroxidase (GPx) as well as the levels of glutathione (GSH) and lipid peroxidation (LPO) were determined. No significant changes occurred in the activities of SOD and CAT. However, the activities of GST and GPx increased in the MC12h group. GSH levels increased in the erythrocyte in the group MC96h. LPO levels increased in erythrocytes of both groups: MC12h and MC96h. GST catalyzes the conjugation of xenobiotics withGSH, thereby facilitating their elimination from cell organelles. The elevated level of GSH suggests a protective role in the MC96h group. The results suggest that after 12 h of MCs injection induced GPx and GST activity and after 96 h the GSH system was activated. However, the antioxidant defenses was not efficient to avoid oxidative stress in the red blood cells as the LPO levels increased in both groups: MC12h and MC96h injected with crude extract of *R. fernandoi*. GSH system and LPO are good biomarkers of acute MCs contamination. Financial support: CAPES Proc. 2276/2011, CNPq/INCT-TA Proc. 573949/2008-5 and CEMIG Proc. GT346.

MO021

Anatomical alterations in rice leaf during senescence and aluminum toxicity

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Aluminum limits the growth and productivity in crop plants. In our present study, we studied the anatomical alterations in aluminum treated rice leaves. The anatomical studies were carried out using Nikon labphoto 2 microscopic Unit. Alittle is known about the anatomical aspects in senescing rice leaf on exposure to Al toxicity. In the present study, two rice varieties i.e., ADT-43, and Pro Agro 6129 were selected to compare and to evaluate histological changes in rice leaves anatomy. The leaf bits were placed in petri dishes of 20 cm diameter and treated with aluminium solution at a concentration of 300μM and distilled water was used as a control. The treatment was exposed for 8 days. We observed that the structures of the leaf ADT 43 when control there was no significant alterations were observed on exposure to 300μm of aluminum, the vascular bundles, Bulliform cells vessel elements and mesophyll tissues are intact. In the control plant the air chambers are reduced in number and the vascular bundles are also much smaller. The bulliform cells are also larger in the control plants. The midrib of Pro Agro 6129 control plant remain intact with wide air chambers, well preserved partition filaments and large and prominent vascular bundles and distinct mesophyll tissue. The bulliform cells are distinct. In the Pro agro 6129 variety there is a general deformity of the midrib as well as lamina on exposure to aluminum. The air chambers in the midrib are reduced too. The vascular bundles of the midrib are slightly modified both in structure and measurements. The lamina is very much affected having lost the structure and organization of the mesophyll tissue, bulliform cell of vascular bundle on exposure to 300 μm aluminum, in Pro Agro 6129. On the whole, the results indicate that ADT-43 is resistant for aluminum toxicity and Pro Agro is sensitive rice variety. **Keywords**: Aluminum, Rice, Mesophylls, leaf epidermal, Stomata.

MO022

TIER II. In situ toxicity and ecological assessment of pesticide run-off in the Madre de Dios River and Lagoon, Costa Rica.

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The Madre de Dios river and lagoon (RMD) is a biodiversity rich watershed formed by a system of streams, rivers, chanels and a coastal lagoon communicating with the Caribbean Sea. This area also sustains an important agricultural activity (mostly banana, rice and pinneapple) with intensive use of pesticides which may pose a contamination risk to this ecosystem. We investigated toxicological and ecological effects caused by pesticide run-off from agriculture. The evaluation was carried out from october 2011 to november 2012 at five sites, four along Madre de Dios river and affluents (sites 1 to 4) and one reference site (5) in a stream outside RMD, that receives less pesticides. Acute toxicity to *D. magna* was evaluated in water samples collected from all sites. Fish biomarkers (Cholinesterase, Glutathione S-transferase and Catalase activities and Lipid peroxidation) were measured in juveniles of *Parachromis dovii*, exposed in cages during 48 hr at each site. An Integrated Biomarker Response (IBR) was calculated. Macroinvertebrate community (MC) structure was investigated by placing four artificial substrates (clay bricks) for colonization over one month, per site. After that, organisms were recovered, sorted in the laboratory and identified. Toxicity to *D. magna* was observed only in site 4 in nov-2011. Responses of fish biomarkers were more frequent and consistent at sites 2, 3 and 4 and this pattern was confirmed by the IBR. Sites with higher IBR were also the ones where higher amounts of pesticide residues were detected. Variations in the structure and diversity of MC related to diminished dissolved oxygen and increasing concentrations of NO_3, total Phosphorus and pesticides. Macroinvertebrates structure was clearly different from site 5, which had the highest diversity and the lowest pesticide concentrations. Amongst the other four sites, the MC was most impacted in site 1, with the lowest diversity and the lowest dissolved oxygen of all. Sites 2 and 3 had very similar community compositions while site 4 shared species with all the other sites. Evidences of effects at different biological organization levels (sub-individual, individual and community) were observed during this evaluation. Early responses in fish biomarkers, acute toxicity and changes on MC were asociated with sites impacted by intensive agriculture, habitat degradation and presence of pesticides.

MO023

PREDICTED TOXICITY RISKS OF PESTICIDES USED IN PADDY RICE FIELDS IN THE MEKONG DELTA, VIETNAM, AND COMPARISON BETWEEN DIFFERENT AGRO-MANAGEMENT REGIMES

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Vietnam is one of the leading rice producing countries in the world with the Mekong Delta being one of its most important regions. The intensive pesticide use in rice cultivation has led to a severe pollution that presents a potential toxicity threat to the environment and public health. There have been several attempts of reducing farmers' pesticide use, such as Integrated Pest Management (IPM) programs. Rice-fish culture is also practiced among many farmers in the Delta and comes with many benefits; besides reducing the economic risks of the farmer it can provide a number of ecosystem services. Integrated rice-fish farming is, however, likely to be more vulnerable to chemical pollution as fish are stocked directly in the trenches of the rice fields. In this study the pesticide use of rice and rice-fish farmers of two provinces, with and without previous IPM training obtained from interview studies was compared using the first-tier risk assessment model. In combination with higher-tier models, worst case application scenarios obtained from PRIMET and risks to aquatic organisms could be identified, in this case with a particular focus on cultivated fish. The results show that there were major differences in risks between the two provinces Can Tho and Tien Giang. The pesticide management of Can Tho farmers had significantly higher acute toxicity risks, mainly due to the use of pyrethroids and organophosphates. Tien Giang farmers were generally using less toxic pesticides such as neonicotinoids. The highest risks were found among conventional rice farmers in Can Tho, indicating that they are able to use more pesticides, as they do not have to consider negative effects on fish. This study shows that the PRIMET model was a useful tool to perform risk assessments on rice farmers' pesticide use in order to understand risks for the surrounding aquatic environment. Many farmers in the area have abandoned integrated rice-fish farming and replaced it with two seasons of rice farming and one season with only fish. This is likely to be a result of pesticides' negative effects on fish in the integrated system. A comparison between the ecological risks following different management regimes and current pesticide compounds used is discussed in order to provide best possible protection of fish and other non-target aquatic organisms.

MO024

Assessment of ecological risks of pesticide use in banana and pineapple cultivation on aquatic ecosystems in Costa Rica using the PERPEST model.

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Costa Rica is a major agro-economy and one of the largest producer of banana and pineapple. These crops are among the world's most pesticide intensive with a total pesticide charge of ca. 45 kg and 30 kg active ingredient per hectare and year for bananas and pineapple respectively. The pesticides may pose a severe contamination risk for Costa Ricas' rich wildlife, particularly in streams and watersheds located downstream from the plantations. In a project called TROPICA (Tiered Ecological Risk Assessment in Costa Rican Agriculture) we gathered data on land use (GIS), pesticide applications (interviews), and pesticide residue concentrations in water and biota samples (GC-MS) in streams of the Limon Province, in the Caribbean Lowlands of Costa Rica in order to assess the toxicity risks from these pesticides on native wildlife. Here we present the results of a risk assessment made using a model called PERPEST. PERPEST is based on a case-based reasoning (CBR) approach, built on data extracted from freshwater mesocosm and microcosm experiments in order to predict the toxic effects of a particular concentration of a pesticide on different taxonomic groups, i.e. crustaceans, fish, and algae. When the effect of a particular concentration of a pesticide has to be predicted, the PERPEST model searches for analogous situations in the database based on relevant toxicity characteristics of the compound, exposure concentration and type of ecosystem to be evaluated. This allows the model to use information on other pesticides for predicting effects of a particular pesticide. In this study, the latest version of the PERPEST model was used, which also allows to evaluate mixtures of pesticides. Hence for each sample taken at each site an overall estimate of the ecological risks was obtained. Results are presented in terms of ecological risk values to various native taxonomic groups and discussed in relation to the pesticide use in the production of bananas and pineapple in the region.

MO025

Additive effects of herbicide and elevated temperature in the branched coral Acropora formosa - Nha Trang, Vietnam.

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Coral communities are currently experiencing global declines in abundance, distribution and diversity. Despite vast interdisciplinary work, few studies have attempted to describe combined effects of environmental stressors (i.e. marine pollutants) and increased temperature on circumtropical reefs. An experimental study was designed to investigate effects of the herbicide Glyphosate and elevated temperature on the tropical staghorn coral *Acropora formosa*, collected in the bay of Nha Trang in November 2012. Combined effects were investigated by means of physiological measurements describing fluctuations in photosynthetic capacity, i.e. gross primary production, dark respiration, chlorophyll *a* content, zooxanthellae densities and coral bleaching using a customized colorimetric method. Fragments of the staghorn coral *A. formosa* were adversely effected by the combination of elevated temperature (+3°C), compared to *in situ* water temperature of 28°C and high Glyphosate exposure (12.0mg·L^{-1}). Significant additive effects of temperature and herbicide were observed both for chlorophyll *a* and for two photometric variables (MIG_E and MIG_{RE}) developed to describe changes in coral physiology through alterations in color by means of digital imagery. The results of this study add further weight to the notion that combined stressors poses a greater threat than earlier recognized. Coral communities within tropical and sub-tropical 'latitude belts' are currently already in close proximity of their upper thermal limits and are additionally exposed to land-use malpractices through increased riverine discharges of suspended particles and pollutants. Conservational policies need to be devised to counteract this course of events. The decrease of chlorophyll *a* and the increase of color loss suggest that coral bleaching is related to the concentration of photo pigments and quantifiable by colorimetric methods. The significant effects of bleaching described by MIG_E and MIG_{RE} respectively show that (1) deleterious effects on coral physiology occur during combined exposure of temperature and herbicide and (2) that there is a relationship between color, zooxanthellae densities and chlorophyll *a*. Measurement of non-intrusive nature, such as the digital image processing method used in this study, should be encouraged and developed further in order to quantify important physiological variables, e.g. chlorophyll *a* content and zooxanthellae densities.

MO026

Oceanic transport of Perfluorinated compounds into Antarctic waters, and the influence of the Antarctic Circumpolar Current

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Despite the apparent pristine conditions and remoteness of Antarctica, persistent organic pollutants have been detected in the region since the 1960s. Today a large variety of organic pollutants can be found in the Antarctic environment and its subsisting species. Unlike the more traditional persistent organic pollutants, perfluorinated compounds (PFCs) such as perfluorooctane sulfonate (PFOS) and other poly- and perfluorinated alkyl substances (PFAS) tend to have very low volatility so are less prone to long-range atmospheric transportation. These types of compounds however readily dissolve in water when in ionic form and appear to accumulate in the world's oceans. As a result of this, one of the primary transport mechanisms for PFCs is believed to be through the oceanic currents. These oceanic currents, similarly to atmospheric currents, are believed to transport these towards compounds the poles where they may accumulate. In the Southern Ocean, the Antarctic Circumpolar Current is major physical feature presenting a barrier for direct north-south transport of surface waters. It is assumed that this barrier is also currently serving to inhibit bulk transfer of hydrophilic pollutants such as PFCs from higher latitude into the water surrounding the Antarctic continent. This hypothesis was supported by recent evidence indicating that Antarctic species, that cross the polar front zone to forage have detectable PFC burdens, whilst those who are restricted to south of the Antarctic Circumpolar Current do not. The barrier however is not impervious, with continuous exchange of older upwelling water bodies and some surface water exchange occurring, presenting the possibility of long term bulk input via this pathway. In order to investigate levels of PFCs in Southern Ocean surface waters and ascertain the role of the Antarctic circumpolar current in mechanistic transfer of pollutants south of the polar front zone, water samples were collected along longitudinal sampling transects from Hobart to the Antarctic Continent in 2011 and 2012. Two litre samples were collected every half to one degree of change in latitude. The preliminary results from this investigation indicate levels of PFOS as well as other PFAS within the southern ocean are currently at levels in the parts per thousand. The trends and patterns of detection are

further interpreted within this presentation.

Marine and coastal ecotoxicology and risk assessment (P)

MO027

Trace element bioaccumulation in rope-grown Mytilus galloprovincialis: knowledge update

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Numerous trace elements (TEs) can be considered as potential pollutants of the environment, their mining productions and industrial uses increasing worldwide. Their monitoring can be achieved through the use of bioindicator species, such as the Mediterranean mussel Mytilus galloprovincialis (Lamarck, 1819). That species has been widely used to monitor the chemical pollution of coastal ecosystems by Cr, Ni, Cu, Zn, Cd, Pb, As, Ag and V. Conversely, environmental levels of Be, Al, Fe, Mn, Co, Se, Mo, Sn, Sb and Bi have been little or not monitored so far in mussel watch programs. Bioaccumulation processes of these 19 TEs in rope-grown M. galloprovincialis purchased from a salt pond with good chemical water quality were thus investigated in the present study.\nMussels efficiently accumulated the 19 studied TEs. Bioaccumulation processes were driven by numerous mutually dependent biological parameters such as the mussel size and flesh weight, the sex and the reproductive status and the body compartment considered. TE bioaccumulation was a power function of the mussel soft body dry weight; total contents linearly increased with the shell length. Small-size mussels overall concentrated more TEs, with a high inteindividual variability, consequently influencing the modelling of their bioaccumulation in the whole rope population. Although a large range of rope-grown M. galloprovincialis sizes can be used for monitoring purposes, one will thus take care not to use extreme size individuals. The influence of gametogenesis in determining female body higher TE concentrations prior to spawning could not be neglected and varied depending on the element. TEs were preferentially accumulated in the hepatopancreas, except for Zn, Se, Cd and Mo, more concentrated in gills. Gametogenesis did not influence TE distribution between body compartments, but likely diluted their concentrations as a direct consequence of massive reproductive tissue production.\nSo, results from the present study underline the potential use of M. galloprovincialis in the biomonitoring of numerous little studied TEs and give some insights into the decisive role played by some relevant biological parameters in bioaccumulation processes of the 19 investigated TEs in rope-grown mussels.

MO028

Accumulation of organotins and perfluorooctane sulfonate in wharf roach (Ligia exotica Roux) and its ability to serve as a biomonitoring species for coastal pollution

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In this study, we measured the accumulation of tributyltin (TBT) and perfluorooctane sulfonate (PFOS) in wharf roach (*Ligia exotica* Roux) and examined the species' ability to be used for TBT and PFOS biomonitoring in coastal environments. In an exposure tests, wharf roach were exposed to TBT or PFOS via diet for 2 d. TBT and PFOS were accumulated in wharf roach. The concentrations of these compounds gradually decreased during the depuration period, but they were still detected 12 d after exposure ceased. The biological half-lifes of TBT and PFOS in wharf roach were estimated to be about 4 d and 11.9 d. In a field study conducted in 2011–2012, wharf roach were collected from 15 coastal sites in Japan and 3 sites in Manado, Indonesia. TBT was detected in both Japanese and Indonesian samples. The highest concentration of TBT was found in wharf roach collected at Bitung ferry port, Manado (57.9 ± 16.5 ng/g), which is close to a shipyard, and the highest concentration at a Japanese site was 12.3 ± 6.2 ng/g. However, PFOS was detected only in Hakata and Tarami ports. Thus, we were able to detect organotins and PFOS in the coastal environments by testing wharf roach, suggesting that *L. exotica* might serve as a good bioindicator for monitoring organotin pollution.

MO029

Metal interaction between the mussel Bathymodiolus azoricus and the polychaete Branchipolynoe seepensis from the hydrothermal vents: endosymbiosis or parasitism?

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Hydrothermal vents are deep-sea environments characterized by high pressure and temperature, as well as high concentrations of heavy metals, sulphides and methane. The vent mussel *Bathymodiolus azoricus* is the dominant species at most sites along the Mid-Atlantic-Ridge, with the capacity to accumulate high levels of metals in its tissues. The commensal polychaete *Branchipolynoe seepensis* is commonly found in the palleal cavity of these mussels, where the polychaete is known to clean the gills surface from particles in excess. Nevertheless, their biological interaction is not yet clear, especially considering metal uptake and bioavailability. So, the main goal of the present work was to understand the association between both species, in a metal bioaccumulation perspective. Mussels *B. azoricus* were collected in August 2013, during the mission BIOBaz, in two sites from the hydrothermal vent Lucky Strike (Eiffel Tower and Monteségur) and one site from the hydrothermal vent Rainbow (PL07). Mussels were dissected and tissues (gills, digestive gland and mantle) and polychaetes (whenever present) were separated, frozen in liquid nitrogen and stored at -80ºC. Metal concentrations (Cd, Cu, Zn, Ni, Fe, Mn, Ag and Co) were determined in total and subcellular fractions of mussel tissues, as well as in whole soft tissue of polychaetes by atomic absorption spectrophotometry. Additionally, a biomarker of metal exposure, (metallothionein-MT) and a biomarker of oxidative damage (lipid peroxidation-LPO) were determined in mussels and polychaetes tissues. The results show different metal concentrations in mussels and polychaetes between sites and subcellular fractions (higher in Rainbow), reflecting inter and intra variation of water chemistry between hydrothermal sites, as well as the ability of these species to regulate their intracellular metal levels (as also shown by MT levels). LPO was higher in mussel gills followed by the digestive gland and mantle, especially in mussels from Rainbow. As for polychaetes, the same tendency was observed, with higher LPO in organisms collected in Rainbow compared to Lucky Strike, while in Menez-Gwen no polychaetes were found. Overall, the presence of the commensal polychaete in gills of *B. azoricus* seems to be an adaptation to the high metal concentrations in the water column near vent sites.

MO030

Acute and subacute responses of Crangon crangon (Crustacea) to ocean acidification due to CO2 injection

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Carbon Capture and Storage (CCS) business is expanding worldwide. During CO2 injection into subsea rocks the surrounding water is threatened by acidification. Moreover, leackage from rocks might contribute to uncontrolled pulses of acidification of seawater. The aim of the project is to identify sensitive marine indicator species and study their behavioural responses to acute and subacute exposure to low pH by CO2 injection. *Crangon crangon*, brown shrimp, is an edible widely spread species. Two different scenarios were tested: (1) stepwise small pH downshifts (0.2 units) from pH 8 to pH 6, (2) continuous exposure to low pH (pH 6.5, 3 days). The experiments were performed in a flow-through system placed in a climate room, the behaviour of the shrimp being recorded quantitatively and continuously with the Multispecies Freshwater Biomonitor (MFB). Shrimp responded rapidly (within 20 Min.) and sensitively to small pH-downshifts in both a stepwise and a continuous regulation with significantly increasing locomotory activity (avoidance). Shrimp also responded to constant low pH with (1) increased avoidance, (2) increased nocturnal activity, (3) post-exposure increased search time for food (optimal foraging theory) and (4) subsequent acclimation to generally low activity towards the end of exposure. In conclusion, *C. crangon* is a suitable indicator species for ocean acidification with rapid, sensitive behavioural responses, which can appropriately be recorded in the MFB in marine monitoring programs. The project is funded by the Research Council of Norway CLIMIT program (project No. 215637). Research work is carried out at the International Research Institute of Stavanger (IRIS), Stavanger, Norway.

MO031

Long term toxicity studies with marine species

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A long term toxicity evaluation was conducted on marine species: algae (*Phaeodactylum tricornutum*), mollusks (*Tapes philippinarum*), crustaceans (*Artemia franciscana*) and fish (*Dicentrarchus labrax*), exposing them to Diethylene glycol (DEG). This is an additive used during oil and gas exploitation process to prevent hydrate formation and corrosion events. It may be released into the sea by the main discharge (Produced Formation Water, PFW) of oil/gas platforms. A full DEG ecotoxicological characterization is required according to Italian law. In literature are reported acute toxicity studies with marine species (bacteria, rotifers, crustaceans, mollusks and fish) but not long term exposure results. The aim of this study is to determine the long term effects of DEG for marine organisms and then to integrate these results with previous acute toxicity studies, to verify if the current DEG threshold value (3.5 g/l) is safe for the marine PFW discharge.

MO032

Combined effects of microplastics and adsorbed contaminants on marine microalgal photosynthesis and growth

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Microalgae play a key role in aquatic ecosystems due to their position at the base of the food chain and contamination at this level could therefore result in unfavourable effects on higher trophic levels. Hence, there is an increasing concern about the potential effects of microplastics on microalgae. These microplastics, synthetic polymers with a diameter smaller than 5 mm to the nanoscale range, have a widespread occurrence and negative effects on different trophic levels have been described. The effect on microalgae can be direct, as adhesion of the plastics to the algal cell walls might block the light needed for photosynthesis, as well as indirect as additives and adsorbed contaminants may leach from the polymers, causing toxic effects. It has been demonstrated that nanoplastics (20nm) can adsorb to microalgae, thereby hindering photosynthesis, but the effect of the larger microplastics on microalgae is still unknown. Therefore, the aim of this study was to determine the effect of microplastics on microalgal photosynthesis and growth. To this purpose a range of marine microalgal species were exposed to different sizes of microplastics (polystyrene) to determine if the effect is species and/or size specific. As microplastics have the ability to both sorb and leach POPs and other contaminants, thereby potentially affecting their bioavailability, the interaction between microplastics and a model herbicide was studied. To this purpose the water was spiked with the photosynthetic inhibitor and microplastics and their joint effect on algal photosynthesis and growth was assessed. This work was funded by the European Union Seventh Framework Programme under grant agreement No 308370 (CLEANSEA project).

MO034

Metals and other elements in tissues of wild fish from fish farms and comparison with farmed species in sites with oxic and anoxic sediments

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The behavior of some metals in the marine environment changes when redox is altered, occasionally resulting in changes in bioavailability. Around organically enriched, highly reducing fish farms, the bioavailability of metals is likely to be low due to the presence of metal-binding phases in sediments, such as organic material and sulphides. However, metals bound to the sediment might pose a threat to aquatic biota either through leaching into the water-column by direct contact or through consumption of contaminated benthic organisms. Wild fish assemblages around fish farms are often exposed to conditions that are unusual for the Mediterranean, such as the hypoxia in farms established at shallow, poorly flushed sites with reducing sediments. The biology and physiology of wild fish may be affected as they become farm effluent feeders, feeding on commercial pellets and subsequently altering their fat deposition and fatty acid composition. Furthermore, recreational and commercial fishing in the vicinity of fish farms is a common phenomenon in Greece. Therefore, it seems that metal and other element determination in farmed and wild fish around fish farms is important in human health risk assessment. Metal accumulation in different fish species may be affected by many factors such as size, sexual maturity, seasonal changes, feeding habits, trophic level, water quality and environmental contamination. In the present study, farmed fish and wild fish aggregating in the vicinity of four Mediterranean fish farms with different environmental conditions were sampled. Levels of metals (including As and Se) were measured in the muscle, liver, gills, bone and intestine. The wild fish from sites with anoxic substrata accumulate metals (including As and Se) from the ambient habitat in their gills whereas those from sites with oxic substrata concentrate these elements through their diet in their intestine. Tissues of wild fish aggregating around farm cages accumulate a greater number of these elements and with higher concentrations than farmed fish. Habitat, diet, ecological needs, fat content of fish, and protein expression may play an important role in these element differences between fish species. Fe in flathead grey mullet, As in surmullet, rainbow wrasse, grey gurnard and picarel and Hg in bogue may pose a risk for human health. Farmed and wild fish are good sources of P, K, Cr and Se while flathead grey mullet, picarel and comber are excellent sources of Ca and Se.

MO035

Metals in benthic macrofauna and biogeochemical factors affecting their trophic transfer to wild fish around fish farm cages

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Macroinvertebrates may accumulate metals from their environment through various pathways, including water, diet and/or sediment, through respiratory and digestive surfaces. However, the effectiveness of metal uptake from these sources may vary according to ecological needs and metabolism of animals and also contamination gradients in water, food and sediment as well as other factors such as salinity, temperature and interacting agents as well as species-specific physiological attributes. Furthermore, metal accumulation may differ according to the ability of macrofaunal taxa to tolerate environmental disturbance. Benthic macrofauna behavior can also influence the pathways, rates and relative balance of sedimentary biogeochemical cycles, mainly through their bioturbation activities. It is known that sediments beneath and close to fish farms are enriched with metals and other elements because of either sedimentation of metals contained in fish feed and fish faeces or due to changes in metal behavior related to modification of environmental conditions. Wild fish are often attracted by fish farms feeding on unused feed pellets but also on benthic invertebrates. In the present study, benthic macroinvertebrates and wild fish aggregating in the vicinity of four Mediterranean fish farms were sampled. Concentrations of metals and other elements were measured in macrofaunal taxa and in fish tissues (muscle, liver, gills, bone, gonad, stomach, intestine, stomach content). Biological and geochemical characteristics play an important role in metal accumulation in benthic invertebrates, and consequently in metal transfer to higher trophic levels. Macroinvertebrates accumulated lower concentrations of most metals and elements than their respective sediment, except As, P, Na, Zn and Cd. Elemental concentrations of benthic organisms increased with increasing sediment metal content, except Cd, and with % silt, refractory organic matter and chlorophyll-*a* of sediment due to the influence of sediment geochemistry on metal bioavailability. Tolerant species were found to accumulate higher concentrations of most metals and elements, except for Cd, than equilibrium species. The ecological and morphological characteristics of the benthic invertebrates can affect the bioaccumulation of metals and elements in macrobenthos. Hg and P were found to increase their concentrations from zoobenthos to of wild fish aggregating around fish cages feeding on macrofauna.

MO036

Contaminants from boatyards - a risk to the coastal ecosystem

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The contamination in pleasure boat yards has been investigated. Measured concentrations of copper, zinc, lead, mercury, cadmium, tributyltin (TBT), the 16 most common polycyclic aromatic hydrocarbons (∑16 PAHs) and the seven most common polychlorinated biphenyls (∑7 PCBs) from investigations at 34 boat yards along the Swedish coast have been compiled. The maximum concentrations were 7 700 for Cu, 10 200, for Zn, 40 100 for Pb, 188 for Hg, 18 for Cd, 107 for TBT, 630 for carcinogenic PAHs, 1 480 for ∑16 PAHs and 3.8 mg/kg DW for ∑7 PCB; all 10-2000 higher than the Swedish environmental qualitative guidelines. In addition, the mean of the median values found at the 34 places shows that the lower guidance value for sensitive use of land was exceeded for the ∑7 PCBs, carcinogenic PAHs, TBT, Pb, Hg, and Cu by a factor of and 380, 6.8, 3.6, 2.9, 2.2 and 1.7, respectively. The even higher guideline value for industrial use was exceeded for the ∑7 PCBs and TBT by a factor of 15 and 1.8, respectively. TBT, PAHs, Pb, Cd and Hg are prioritized substances in the European Water Framework Directive (WFD) and should be phased out as quickly as possible. Because of the risk of leakage from boatyards, precautions should be taken. The high concentrations measured are considered to be dangerous for the environment and human health and highlight the urgent need for developing and enforcing pleasure boat maintenance guidelines to minimize further soil and nearby water contamination.

MO037

Impact of boat maintenance on biofouling

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Biofouling of boat hulls is a natural process that can have detrimental economic and environmental effects because it leads to increased fuel consumption and CO_2 emissions and facilitates the transport of invasive species to new habitats. The extent of biofouling (expressed as dry biomass) on 102 leisure boats in Stockholm, Sweden was assessed in relation to different boat maintenance methods such as the use of copper, zinc or biocide-free paints and the use of boat washers. The influence of other factors such as sailing time, boat age or boat position in the harbor was also investigated. No significant difference was found in the amount of fouling on boats using copper, zinc or other biocide-free paints. This result is rather surprising because it is not in accordance with the common knowledge that copper paints are the most efficient antifouling paints currently available. Moreover, the common belief that sailing often reduces the amount of biofouling does not apply in our study, because there was no relationship between the sailing time and the degree of fouling. As regards the different areas of the boat hull, the stern was affected by biofouling to a significantly higher extent than the port or the starboard side. In addition, boats which have been positioned with the stern towards East have significantly higher fouling than those positioned towards North or West. Since the

light conditions affect the growth of photosynthetic organisms (which were the predominant fouling organisms in our study), we recommend considering the boats' position in harbors for minimizing fouling. Moreover, boats that have used boat washers had significantly lower fouling at the end of the season so we encourage the use of this environmentally-friendly maintenance method. These results suggest that the maintenance habits ought to be reconsidered and that the use of toxic compounds in antifouling paints may be unnecessary for leisure boats in the northern part of the Baltic Sea.

MO038

Lethal and sub-lethal effects of aniline to the copepod Tisbe battagliai: simulation of Hazardous and Noxious Substances (HNS) spill profiles

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Hazardous and Noxious Substances (HNS) are chemicals, other than oil, which have potential hazards to human health or to other organisms. Over the last decades transportation of HNS by sea has increased significantly so emphasizing the need to be prepared to respond to incidents involving HNS. The European project ARCOPOLplus recently identified 23 HNS as having high likelihood of being present in martime transport incidents suggesting these be the focus of efforts to fill data gaps. This study therefore considered aniline, one of the 23 chemicals listed as priority for improving existing data. The volume of chemical spilt at sea and its behaviour (e.g. whether it dissolves or evaporates) and location (e.g. open sea or nearshore) are major factors influencing extent of any impact. Chemical modelling can predict the dispersion and fate of a chemical during a spill and this can be linked to toxicity data to predict likely impact. However toxicity data is usually derived from constant exposure studies and under natural conditions this rarely occurs. This work investigates the acute and chronic toxicity of aniline using representative spill profiles. The copepod *Tisbe battagliai* was chosen as test species because of its sensitivity and importance in the marine food webs. Tests included short exposures (1 and 2 hours), simulated varying concentration spill profiles (based on modelling data) and constant exposures. Different life stages of Tisbe were used to obtain acute and sub-lethal effects data. Results indicate that aniline does not have prolonged effects even after exposure to relatively high peak concentrations providing exposure duration is short e.g. 1-2 hours. Preliminary data indicate realistic spill profiles may have negligible impact for chemicals that do not accumulate or persist due to the relatively rapid dilution that occurs at offshore locations. At near shore locations more significant effects are predicted. This study indicates that use of standard toxicity data could lead to an overestimate of the potential harm caused by a spill. Whilst such a precautionary approach is recommended where there are significant threats to marine resources, more realistic assessments may be of value in predicting actual levels of impact and potential for recovery. *This work has been carried out with support from Defra, Project MERR C5905 and in the frame of ARCOPOLplus project, co-funded with ERDF through the Atlantic Area Transnational Programme*

MO039

Integrated assessment of the chemical environmental state of Cartagena Bay (NW Mediterranean) in relation to marine chemical contamination

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Cartagena bay (SE Spain) was chosen as study case of the NW Mediterranean region to demonstrated the suitability of the integrated marine environmental monitoring of chemicals and their effects proposed by European experts (ICON Project) for the North Sea. Coordinated sampling of key environmental matrices (surface sediment, fish and mussels) was performed in Autumn 2008, considering biogeographical characteristics. The benthic fish red mullet (*Mullus barbatus*) and mussels (*Mytilus galloprovincialis*) were used as target species. Due to scarcity of mussel populations on natural substrates, mussels were transplanted for a period of six weeks in a coastal site located in the bay. Contaminant related-biomarkers of exposure and effects were measured in both target species. Contaminant concentrations were analyzed in sediments and biota but also and sediment-passive samplers. *In vivo* embryotoxicity bioassays were performed using sediment elutriates. Additionally, a battery of *in vitro* gen reporter bioassays covering different mode of action of toxicants were also investigated using extracts of the sediment-passive samplers. Chemical and biological data were assessed against its corresponding assessment criteria, and then integrated over levels of matrix. Assessment was expressed with varying levels of aggregation (contaminants, bioassays, biomarkers of exposure and biomarkers of effects) to graphically represent the proportion of different types of determinants exceeding either level of assessment criteria. Subsequently, data were aggregated further into a single schematic showing the proportion all determinants that exceed assessment criteria in Cartagena bay, using a threshold of 95%

MO040

Hepatic histopathological findings in Ariidae Cathorops spixii submitted to anthropogenic metal exposure in two Brazilian estuaries

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Histopathological lesions are used as biomarkers of contamination by organic and inorganic chemical compounds in the aquatic systems and can reflect chronic effects in sentinel species. Previous work demonstrated the ability of the catfish *Cathorops spixii* to be an efficient bioindicator species for metals contamination. Cananéia estuary is considered an area of environmental protection, and UNESCO World Heritage Site (WHS), and as such, several biomonitoring studies in the São Paulo State, Brazil, use this estuary as a reference environment due to the fact that it shows very little human influence. However, in recent years, several studies have been indicating the presence of some toxic metals, for instance mercury (Hg) and lead (Pb). Santos-São Vicente estuary has a long history of contamination and release of pollutants, for instance metals such as Pb, Cd, Ni, Mn, Cu and Hg as a consequence of different anthropogenic activities such as petrochemical, metallurgical and harbor. In the present work, histopathological lesions in liver were investigated in the Ariidae *Cathorops spixii* from Cananéia estuary and in two sites affected by industrial and domestic sewage disposal, in order to verify the possible impact of the introduction of metals to the local fish species. Fish were collected in Cananéia and in two sites of the Santos São-Vicente estuary subjected to different anthropogenic influence (industrial and domestic sewage disposal). Biometric data (total length –TL- and total weight -TW- of each fish) was obtained and the fish dissected by collection of the hepatic liver to histopathological and metals analysis. Analyses of Hg was performed by FIA-CV AAS and Pb, Cd, Cu, Mn and Ni concentrations were determined by ICP MS. Fish from Cananéia showed higher levels of Mn (80%) and Cu (66%). The most important injuries observed in individuals from the impacted areas (industrial and domestic sewage) were the presence of necrotic areas, hepatitis focal, vacuolization and rupture of blood vessels. The occurrences of lesions were significantly higher in individuals from domestic and industrial area than in fish from Cananéia estuary ($p>0.05$).

MO041

Influence anthropogenic pollution on starfish's Asterias rubens at different level of biological organisation

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There is growing concern that the invertebrate test organisms commonly employed in the field of aquatic ecotoxicology may not be sufficient to accurately screen for the possible deleterious effects of contaminants discharged into the marine environment. The use of echinoderms has been proposed to redress this problem, due to their ecological importance and their evolutionary closeness to the chordates. A suite of biomarkers which operated at different levels of biological organization (sub-cellular, cellular, individual and community level) were identified for use with the common starfish *Asterias rubens* L. *and copepods Scottomyzon gibberum* Scott. Adult females of *S. gibberum* live on starfish body surface of and can induce galls formation. Specimens of the *Asterias rubens* were collected near the Biological station of Lomonosov MSU at Kandalaksha Bay of the White Sea during the summers of 2012-2013 years from 10 points which characterized by different anthropogenic pollution level and ecosystem type. Indexes which widely used in parasitological studies were calculated to characterize population: prevalence, intensity, abundance, aggregations indexes and also copepods location on host body. The population increases from mid-June to late September. Population heterogeneity is shown, which can be attributed to anthropogenic load and ecosystem type. Population of copepods on starfishes increases with depth. Ecotoxicity bioassay was made with 4 different heavy metals composite concentration in salinity 21 and 25.7 ‰ (normal). Micronuclei induction was estimated in coelomic fluid phagocytic cells, which are caused by both chromosome breakages and spindle apparatus dysfunction. Low salinity level case of decrease proliferation of phagocytic cells and bacterial infection. In normal salinity heavy metals cause a dose-dependent increase the number of micronuclei. Habitat reaction (righting behaviour) shows adaptation for influence heavy metals and salinity after 10 days of experiments.

MO042

Early maternal exposure to estrogens cause malformations in eelpout fry

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Recently malformations among eelpout (*Zoarces viviparous*) fry living in North European coastal areas with high anthropogenic input have been observed. The specific chemicals or group of chemicals causing the observed malformations are unknown. In oviparous fish species fry malformations can be induced by exposure to chemicals including endocrine disrupters. The eelpout is a viviparous and stationary fish and maternal exposure to chemicals including endocrine disrupters might explain the fry malformations observed in nature. The aims of the present experiments were to investigate mother-offspring interactions e.g. teratogenic effects upon maternal exposure to 17β-estradiol (E2) and environmental chemicals with known endocrine disrupting effects; the PAH pyrene, the synthetic hormone 17α-ethinylestradiol and 4-*t*-octylphenol and to investigate if a teratogenic window for E2 could be established. Wild pregnant eelpout with newly fertilized eggs were either (1) exposed continuously to 17β-estradiol (E2) (5.7-133 ng/L), 17α-ethinylestradiol (≈20 ng/L), 4-t-octylphenol (≈6.25-50 ng/L) or pyrene (≈ 500 ng/L) for six weeks or (2) exposed to E2 during different weeks of pregnancy to investigate if a teratogenic window for E2 could be established. None of the chemicals influenced the survival of the females. Plasma levels of E2 and the yolk protein precursor vitellogenin were increased in mothers exposed continously to E2 and an increased abundance of fry malformations was observed at the highest E2 concentration. If exposure takes place within the first two weeks of pregnancy ovarian function and fry development is severely affected. As eelpout are fairly stationary during their pregnancy individual populations could be differentially influenced as the local exposure scenarios are different and most likely vary from year to year.

MO043

Effects of 107Ag and 63Cu stable isotope sublethal exposure in oysters Crassostrea gigas using cell and tissue level biomarkers

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Marine bivalves such as oysters are known to be good sentinel organisms. Their sedentary way of life and their ability to accumulate pollutants with little metabolic transformation make them ideal organisms for this purpose. Many marine pollution monitoring programs have used oysters to obtain chemical and biological data. Previous studies have demonstrated that cell and tissue level biomarkers are reliable and sensitive tools to assess organisms and environmental health status. Silver and copper are both elements present in the environment that in high concentrations impair biological processes and affect environmental health status and even human health. In order to study the biological effects produced by these metals and their accumulation kinetics and pathways, a 28 d laboratory experiment was carried out. Oysters were exposed directly to a range of environmentally relevant concentrations of silver and copper stable isotopes (^{107}Ag and ^{63}Cu). The use of stable isotopes allowed precise and rapid tracing metal accumulation in five different tissues. Different endpoints were measured in oysters at different levels of biological complexity including induction of metallothioneins, alterations in lipofuscin and lipid contents, metal accumulation and distribution in tissues and target cells, histopathological alterations at tissue level, and changes in organism condition and mortality rates. Results indicated higher mortality in oysters exposed to high concentrations of Ag and the combination of Ag and Cu together with a general decrease in the condition index after 20 days of exposure. Histological examination and quantification of alterations in the digestive gland indicated a higher digestive gland atrophy and over time tissue degeneration in animals exposed to high Ag and Cu concentrations. A similar pattern was observed for histopathological lesions. Moreover, in the more affected oysters a progressive arrest of the gamete development occurred. Autometallographical screening for metal ions indicated the presence of metals mainly in the gill epithelium, the digestive cell lysosomes and in the basal layer of the digestive tubule epithelium of oysters exposed to Ag and Cu. In general, obtained results indicate a close relationship between metal exposure at environmentally relevant concentration levels and physical degradation of oysters. Acknowledgements: Funded by the Basque Government (grant to Consolidated Research Groups; GIC07/26-IT-393-07).

MO044

Intertidal biota and the rock shell populations after accidents of Fukushima Dai-ichi Nuclear Power Plants

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To investigate possible adverse ecological effects caused by accidents of the Fukushima Dai-ichi Nuclear Power Plants (1F) accompanied with big earthquakes and Tsunami in March 2011, we conducted field surveys at intertidal zones of 43 sites along the coastal line of eastern Japan from April to August 2012. The number of species of intertidal biota seemed to get smaller as the site was close to 1F. No rock shell (*Thais clavigera*) specimens were collected at 8 sites of Fukushima prefecture, which were located around 1F. Because the rock shell specimens were collected at many sites in Miyagi and Iwate prefectures, where enormous Tsunami attacked, it is unlikely that smaller number of intertidal species and no rock shell specimens around 1F were caused by Tsunami. Contaminated cooling water leached from the nuclear reactors to the sea may have given any impacts to intertidal biota including the rock shell populations around 1F.

MO045

Application of European C14 method (OECD 215) on early life stage fish growth response to marine species Dicentrarchus labrax

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C14 Method as described in the EU Regulation 440/2008 on juvenile fish growth toxicity test is designed to assess the chronic effects of chemicals on freshwater species. However, the use of a marine species widely distributed and well known as the sea bass can be considered more appropriate to assess the impact of dispersed chemical substances in the sea. The purpose of this study is to analyze the adaptation of the C14 Method to the marine species *D. labrax*. Toxicity tests were carried out exposing *D. labrax* to sodium dodecyl sulfate (SDS). This is an anionic surfactant widely employed in industry, agriculture, and domestic use and therefore it is found in abundance in the environment, particularly in the sea. Adaptations of the method are reported and results of growth response of *D. labrax* to the SDS are showed.

MO046

Evaluation of the detoxification mechanisms of metals in aquatic organisms by characterization of hepatic metallothionein (MT)

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Metallothionein (MT) has been thoroughly used as a biomarker of metals exposure. MT is a low-molecular-weight protein which has many sulfhydryl groups due to the large amount of cysteine in the molecule. These sulfhydryl groups bind a variety of metals and therefore, presumably, make them less toxic to other cellular constituents. However, biochemistry aspects of the protein as isoforms identification and quantification and their specific mechanisms of detoxification in sentinel species are, as yet, weak. Previous data showed that fish, such as the catfish *Cathrorops spixil*, are efficient bioindicator species to metals contamination in coastal aquatic areas under anthropogenic influence, for instance inputs of Pb, Cd, Hg, Ni, Fe, Zn, Cu and Mn. *C. spixil* is the most common catfish in the Brazilian coast and has a feeding habit mainly of materials and organisms upon the sediment, where the availability of contaminants is high. In order to understand intrinsic aspects of the detoxification process of toxic metals in sentinel species in natural and anthropogenic conditions, hepatic samples of *C. spixii* were collected in a non-polluted (Cananeia), as well as one polluted, estuary (Santos-São Vicente) and tested under different assays in order to establish an effective bioanalytical technique to purify the protein like-MT, identify and quantify the specific isoforms and the metal contents in the cell. Therefore, hepatic samples were submitted to ultracentrifugation, thermocoagulation and a chromatographic purification and identification of MT isoforms by size-exclusion-HPLC and anion-exchange-HPLC, respectively. The elution of the protein was made with online UV/Vis detection. Metals in hepatic cytosols were also quantified by HR-ICP-MS. Obtained results showed that the established protocol of bioanalytical technique was effective to purify MT-like protein by SE-HPLC and to identify its isoforms by AE-HPLC. With these analytical strategies, it was possible to identify two kinds of MT isoforms (MT-1 and MT-2) in the *C. spixil*. Fish MT-1 from polluted areas showed a strong linkage with the levels of Cu, Hg, Pb, Fe and Ni suggesting that the MT-1 is an effective biomarker of metal contamination.

MO047

Assessment of the environmental impact of the dumped chemical warfare agents at the Baltic Sea using caged blue mussels (Mytilus trossulus)

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Chemical weapons dumped into the sea after World War II possess growing concern for the marine environment; metal shells of different chemical munitions lying on the bottom are severely corroded and dangerous contents pollute the sediments. Chemical warfare agents (CWAs), such as mustard gas and various arsenic-based compounds (e.g., Clark I and Adamsite) and their degradation products have been detected in noticeable concentrations in sediments at the major dumping sites at the Baltic Sea. Blue mussel caging approach was applied to assess environmental impact of thousands of tons of CWAs at the main dumping site at the Bornholm Basin. Due to the patchy occurrence of the CWAs in the sediments mussel caging method was chosen to deploy the organisms exactly at sites where high CWA concentrations were detected in sediments and to one reference site. Biomarkers representing different biological functions including antioxidant defence, biotransformation, neurotoxicity, lysosomal membrane stability, geno- and cytotoxicity, cellular energy allocation and condition index were investigated. Moreover, tissue concentrations of different CWAs and the possible metabolic

derivatives of these compounds were analysed in mussels together with “classical” contaminants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and trace metals. Further, mussel cages were equipped with salinity, temperature and oxygen sensors to follow the fluctuations in the environmental parameters during the two and half month caging time. Environmental parameters indicated mixing of the near bottom water in the lower water layer where the cages were deployed (at 60 meters). Significant biomarker responses were observed at the two contaminated sites compared to the reference site indicating CWA induced effect on molecular and cellular level; however the possible anaerobic conditions and lower food availability in deep waters led to decreased bioenergetic status of the caged mussels. Results of the still on-going chemical analysis will be employed together with the biomarker responses and environmental data in the multi-level integrated impact assessment of the area.

MO048

Tracking Munitions in Two Coastal Marine Ecosystems Using Stable Nitrogen Isotopes

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2,4,6 – trinitrotoluene (TNT) and hexahydro – 1,3,5 – trinitro – 1,3,5 – triazine (RDX) are munitions compounds used widely at U.S. Department of Defense facilities, including coastal military installations. TNT and RDX are introduced in many countries into coastal marine ecosystem through manufacturing, detonation, and legacy munitions. TNT and RDX and their derivatives are U.S Environmental Protection Agency priority pollutants and have known toxicity for terrestrial and aquatic species. While both TNT and RDX have been shown to persist in freshwater environments and soils less is known of the fate and transport in marine systems. As part of a multi-scale marine ecosystem fate and transport study, a time series of large mesocosms that includes sediments and varying trophic levels of biota were spiked with nitrogen isotope labeled TNT and RDX separately. TNT and RDX bulk concentrations (and associated breakdown products) in seawater and biomass were measured using extraction methods in acetonitrile and GC/ECD. Nitrogen isotope values were obtained using EA/IRMS methods. TNT was found along with 2 primary derivatives 4 amino-dinitrotoluene (4-ADNT) and 2 amino-dinitrotoluene (2-ADNT). The derivatives were measured often 2 times higher than the total TNT in all biota. The ratio of 4-ADNT to 2-ADNT measured in the biota was often found to be 3:1 respectively. In the majority of the biota, the ^{15}N attributed to the munitions continued to rise through the time series. Only 2 to 3 percent of the total ^{15}N in the biota can be attributed to munitions measured in the tissue. When normalized to mass, the macro algae contained 50 percent or more of the total ^{15}N in the system. This large percentage is most likely due to the uptake of ammonium measured in the system. RDX and four main derivatives were measured with MNX the only derivative that was measured with any consistency. The ^{15}N attributed to the RDX was taken up quickly but did not continue to rise over the time series. Approximately only 3 percent of the ^{15}N can be attributed to RDX. The total μmol of ^{15}N normalized to mass was typically equal in each species in the system. In both ecosystem experiments, the TNT and RDX were shown to enter into unknown pathways within the biota. Further studies with amino acids and single organ system analysis are in progress to better understand the fate of the munitions.

MO049

Using stable nitrogen isotope tracer to investigate ecosystem distribution and fate of munitions compounds in marine environments

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The explosives 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are common munitions constituents. Within the contiguous 48 United States, there are approximately 41 active military installations located within the coastal zone. Munitions operations on active facilities consist of storage, loading, packing, and live fire exercises. Several active installations maintain live fire ranges in intertidal and subtidal marine habitats. The number of active bases, combined with decommissioned facilities with historical TNT /RDX contamination, and offshore munitions dump sites indicate the probable likelihood that multiple marine habitats at multiple locations receive exposure to TNT and RDX. Currently there is little ecosystem-level understanding of how munitions compounds behave in marine environments. How fast are these compounds assimilated into various organisms or trophic levels, under natural conditions? How fast are they transferred between trophic levels, mineralized, cycled, or stored? What ecosystem characteristics control the partitioning of these compounds between pathways that remove them, and pathways that store / recycle them? Here we present an approach using ^{15}N isotope labeled munitions compounds that may provide resolution to some of these ecosystem-scale questions. Theory and application is presented as well as results of multi-trophic level experiments that demonstrate the efficacy of using this approach to resolve magnitudes of compound breakdown vs accumulation in biomass. Additional keywords: Stable isotope, nitrogen, munitions, ecosystem, marine

MO050

Tracing the cycling and fate of the explosive 2,4,6-trinitrotoluene in a simulated sandy coastal habitat with a stable isotopic tracer, 15N – [TNT]

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TNT is produced and used in massive quantities worldwide. In the United States, TNT contamination has been observed in ground and surface water, as well as in soils. Even more alarming is the quantity of unexploded ordnance (UXO) dumped directly into the ocean over the last 100 years. Despite the extensive studies that exist on the behavior of TNT in the environment, including solubility, transport, transformation, remineralization, and biological uptake, several factors limit our understanding of the ultimate toxic potential of this compound in marine systems. Specifically, it is difficult to predict how these individually defined controlling factors will interplay in an environment as physically, biologically and chemically complex as the coastal ocean. Here, we introduce both single pulse and steady-state additions of isotopically labeled ^{15}N-[TNT] into a previously constrained aquaria-scale simulated coastal marine habitat and perform extensive enrichment analysis of both dissolved and particulate organic and inorganic N fractions in order to provide the most complete view to date of the partitioning and the ultimate fate of TNT in seawater. ^{15}N enrichment was observed in all measured fractions, including NO_x, NH_3, N_2, bulk sediment N, suspended particulate N (PN), and N present in biological tissue. The production of reduced derivatives, 2amino-dinitrotoluene (2a-DNT), and 4amino-dinitrotoluene (4a-DNT), was also observed. Principal components analysis (PCA) indicates two distinct reaction pathways, the first proceeding through reduction to reduced derivatives, stripping off of ammonia functional groups, and subsequent denitrification. The second, as yet undefined, is initialized through surface sorption to suspended particulate matter (SPM). In fact, the concentration of SPM is shown to control the overall breakdown rate of TNT in the system. A mass balance of measured ^{15}N fractions accounts initially for 80% of all introduced ^{15}N, but only 20% at the end of the experiment. We provide evidence through PCA and correlations with reduced derivatives that the “missing” ^{15}N represents the buildup of an unmeasured derivative.

MO051

Toxicity of PAHs in Mytilus galloprovincialis at different stages of gametogenesis measured by physiological biomarkers

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Large scale marine monitoring programs have some difficulties in the assessment of the effect of pollution in the biological responses of the organisms, mainly due to the high temporal and spatial variability of marine ecosystems. In this context, gametogenic cycle has to be considered due to its influence on the biomarkers normally used as indicators of pollution. The gonadal development of the wild mussel (*Mytilus galloprovincialis*) used as a sentinel species in the N-NW Spanish marine pollution monitoring Program, takes place between late autumn and winter and spring where main spawning occurs. During late summer and early autumn, there is a storage of reserves that will be used for the next gametogenesis. The aim of this study was to evaluate the effect of the reproductive status of the mussels on their response to the polycyclic aromatic hydrocarbon (PAH) fluoranthene (FLU), measured by several physiological biomarkers as clearance rates, absorption efficiency and respiration rates which can be integrated in the scope for growth SFG index. Exposure of mussels to FLU was carried out twice: when the mussels are in resting time (September-October) and when the gonads are completely full (March). Mussels were exposed to two nominal FLU concentrations (3 and 60 $\mu g\ L^{-1}$) for 3 weeks. Then, mussel physiological rates were measured under the same standardized laboratory conditions (temperature and food concentration) for both reproductive stages. Biological characterization of mussels from a biochemical, histological and anatomical point of view was performed.

MO052

Comparative study on PAH metabolites in cod (Gadus morhua) from the Baltic Sea to Greenland

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Polycyclic aromatic hydrocarbons (PAH) are important environmental contaminants which may lead to reproductive effects or formation of liver tumours and associated lesions in fish. Therefore PAH and their metabolites are part of international monitoring programmes and recommendations. In the light of the

European Marine Strategy Framework Directive monitoring data and assessment criteria are brought together for evaluating the environmental status in marine and coastal regions. Low contaminated reference areas are needed to calculate background assessment criteria. Cod (*Gadus morhua*) is a fish species widely distributed in northern European waters and therefore a suitable object for environmental assessments on a big regional scale. Cod samples collected between 2006 and 2012 in the western Baltic Sea, North Sea, Barents Sea, Norway and Greenland were included in the present study. To assess the PAH exposure of fish, the concentration of the main metabolite 1-hydroxypyrene was determined in bile by HPLC with fluorescence detection. The results show that PAH metabolites can be used to create a spatial overview on PAH contamination in northern European marine fish such as cod. Concentrations of PAH metabolites differed according to sampling region. Lowest concentrations were found in cod caught close to Greenland suggesting this area as reference region to develop new background assessment criteria.

MO053

Alterations on metals incorporation and oxidative stress endpoints in caged Carcinus maenas reflecting intermittent releases of sedimentary metals - a day-night cycle in a eutrophic system

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Coastal lagoons with symptoms of eutrophication often present low oxygenated waters, particularly during the night. Under such conditions, sediment could release metals to the overlying water. Thus, inhabitant organisms may accumulate metals that were provided from the sediment, which could be on the basis of physiological responses. In summer 2007, a field transplantation experiment was performed with *Carcinus maenas* in three short-term exposures (8 h) along 24 hours, namely: (i) period 1 - between 15:30-23:30, corresponding to a day-night exposure; (ii) period 2 – between 23:30-07:30, corresponding to a night exposure entirely; (iii) period 3 – between 07:30-15:30, corresponding to an exclusively day exposure. The study was carried out in a coastal lagoon with eutrophication symptoms and moderate contamination by metals (Óbidos lagoon, Portugal), addressing particularly a confined area (Barrosa branch – BB) where metals could be released from the sediment at night. Crabs werecaptured at a reference site (Lower lagoon - LL) and transplanted to BB and caged, in order to evaluate the accumulation of metals (Fe, Zn, Mn, Pb, Ni) and oxidative stress responses (CAT, SOD, GR, GST, GSH_t, LPO) in hepatopancreas. Selenium levels were also determined in hepatopancreas due to its antioxidant role as cofactor. The results were compared with a group caged at LL. Crabs transplanted to BB exhibited higher levels of metals in hepatopancreas, particularly Zn (after period 1), as well as Ni and Se (after period 3), reflecting the higher metal availability at this site. The enhancement of Se levels at BB could be regarded as a protective mechanism against a pro-oxidant pressure. Moreover, as a sign of toxicity, a CAT activity reduction and increased LPO levels were recorded in hepatopancreas of crabs transplanted to BB site (after period 3), matching with the higher accumulation of Ni. Overall, it was demonstrated that the intermittence on metals bioavailability can be detected. Metals accumulation was not completely synchronised with physiological changes or with the peak of metals in water, revealing the complexity of biological responses. Nevertheless, oxidative stress endpoints measured in crab hepatopancreas showed their efficacy as early warning markers and, due to the nature of the biochemical phenomena involved, appear as particularly adequate to detect fluctuations on water quality in a hour time scale.

MO054

Baseline of oxidative stress biomarkers in natural populations of sea anemones in three ecoclimatic marine environments

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Biomarkers have been widely used for the assessment of exposure and/or effects to environmental contaminants. The oxidative stress biomarkers is common used on marine organism to assess the stress levels and effects and damages caused by natural, chemical and/or physical stressors. The sea anemones target species has a wide distribution in the Atlantic and coastal areas where it may be found in both contaminated and pristine areas. It is abundant and plays a key role in coastal ecosystems. Therefore, this species seems to be a good candidate to be used as a sentinel species. It may then be assumed that six co-generic species *Anemonia sargassensis, Anemonia sulcata, Actinia bermudensis, Actinia equina, Bunodossoma canjicum* and *Bunodossoma caissarum* could be a valid tool for evaluation studies of environmental contamination. The main aim of this study is to detect significant basal levels activity GST, GR, CAT, LPO and SOD measurable as a biomarker environment, on natural populations of sea anemones, under environmental stress and sources of pollution in three different climatic scenarios. In order to detect different levels of contamination in the sampling locations, validating the combination of the activity levels of these enzymes with different pollutants sources and verify the spatial and interspecific variations.

MO055

Effect of nutritive condition on antioxidant biomarkers: Consequences for large-scale monitoring programs

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The monitoring of the chemical pollution and their biological effects carried out by the Spanish Oceanographic Institute along the N-NW coast of Spain is based on the study of both the accumulation and biomarker responses in mussel (*Mytilus sp*). This program covers more than 2,500 km of coastline, and is characterized by a great variability of environmental conditions such as food availability and quality, temperature or salinity. In previous works we have found that environmental conditions variability produce spatial differences in mussel nutritional status which seems to mask the biomarker responses to pollution. A laboratory study was designed in order to establish the levels of antioxidant biomarkers in mussels within a gradient of energetic status. Several experimental trophic conditions were simulated by regulating daily food ration from very negative to highly positive energy balances. Levels of catalase (CAT), glutathione transferase (GST), glutathione reductase (GR), and lipid peroxidation (LPO) in the digestive gland were used as biomarkers of oxidative stress. Relationships between mussel body reserves (lipids, proteins and carbohydrates) and levels of those biomarkers have been established. Comparisons between biomarkers levels found in the field study at N-NW coast of Spain with those from laboratory simulations were used to assess the effect of the mussel nutritive condition on these biomarkers. In general, nutritive condition is clearly affecting the level of the antioxidant biomarker.

MO056

The effect of pharmaceutical compounds on the larval development of the estuarine shrimp Palaemon longirostris

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The most frequently detected pharmaceuticals compounds (PhCs) in coastal waters comprise the anti-inflammatory and analgesic diclofenac sodium (DS), the lipid regulator clofibric acid (CA) and the fungicide clotrimazole (CLZ). These compounds are on the priority lists of the UK Environment Agency and the Oslo and Paris Commission (OSPAR). We studied mixture effects of CA and DS, and individual effects of CLZ on growth, development and body mass of larval stages of the estuarine shrimp *Palaemon longirostris*. Drug residues found in the aquatic environment usually occur as mixtures. Thus, an accurate prediction of the mixture toxicity of DS and CA could be indispensable for environmental risk assessment. In previous studies on marine larvae of *P. serratus* identified toxic effects of CLZ on larval survival and larval growth, and these effects depended on osmotic and food stress. Not all species should respond to environmental change in the same form; thus, we must also consider the inter-specific life histories to test the potential toxicity effects of CLZ in the estuarine shrimp *P. longirostris*. The larvae were exposed to environmental relevant concentrations (DS: 36-70, CA: 9.6-40 and CLZ: 0.07-0.14 $\mu g\ L^{-1}$) and concentrations that are higher than those measured in nature in order to explore thresholds that lead to sublethal effect (DS: 700 ; CA: 400 μg and CLZ: 4 $\mu g\ L^{-1}$). Larvae were reared at two temperatures (18°C and 24°C) and two salinities (20 and 32 PSU). At environmental concentrations, these PhCs had no effect on larval survival and development of *P. longirostris*.The effects of these PhCs on larvae appeared at high doses. At both experimental temperatures, CA increased duration of development in *P. longirostris* larvae. Toxicity of the mixture DS and CA, increased duration of development at the lowest experimental temperature and reduced number of stages and growth rate. These effects were similar to CLZ effects, but in this case the number of stages had a significative reduction. DS, at high doses and warmer waters, decreased growth rate and larval body mass. In particular, CLZ, CA and the mixture of CA and DS increased intermoult duration required to reach the first juvenile stage. Acknowledgements This work was carried out while E. González-Ortegón was MC Pstdoctoral Fellowship from the EC.Furhter financial support was provided by SCARCE (Consolider-Ingenio 2010 CSD2009-00065).

MO057

Effects of tralopyril, triphenylborane pyridine and capsaicin on zebrafish

embryos (Danio rerio)

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Biofouling is associated with problems such as the deterioration of diverse aquatic equipment and installations, the dissemination of invasive species or increased fuel consumption by ships due to frictional drag. Antifouling (AF) biocides are added to paints to boost their efficacy for biofouling prevention. These antifouling paints (AFP) are economically highly relevant. However, often they were found to harm the environment. This obvious conflict is far from being solved. Restrictions in the use of AF biocides imposed by the Biocidal Product Regulation (BPR, Regulation EU No. 528/2012) are resulting in an increased need for environmentally safer alternatives. Such substitutes are expected to maintain a good AF performance, but at the same time to undergo a rapid transformation to less toxic products once released into the water. Moreover, they should have low toxicity to non-target organisms as well as low tendency to bioaccumulate. The current work investigates the toxicity of emergent AF biocides, tralopyril, triphenylborane pyridine (TPBT) and capsaicin, to the early life stages of the non-target model organism, zebrafish. The endpoints assessed were the mortality and the occurrence of sublethal effects. Based on preliminary data, tralopyril seems to be the most toxic among the three compounds as the LC_{50} ranged between 6 and 8 μg/L. For TPBP, LC_{50} is within 500 and 750 μg/L, while capsaicin seems not to induce mortality up to 1mg/L. Sublethal effects included slow heart rate, heart edema, deformations of the axis and non-inflation of the swim bladder. Tralopyril showed a clear dose-response in the inhibition of the swim bladder inflation at 120 hpf (EC_{50} around 2 and 4 μg/L) and seldomly was found to cause lordosis. TPBP also influenced the inflation of the swim bladder, but data are not as consistent as for tralopyril. Moreover, TPBP induced heart edema starting from 100 μg/L and diminished the heart rate at 250 μg/L and above. For capsaicin, the most relevant effect was the increase in the heart rate of embryos exposed to 100 μg/L and above. Since the mode of action of the investigated compounds is incompletely understood, the causes underlying the reported sublethal effects remain unclear. With the ongoing proteomic analysis we hope to reveal differentially expressed proteins that may provide first insights into the molecular mechanisms accountable for these toxic effects.

MO058

Pharmaceutical residues and occurrence of antibiotic-resistant bacteria in Hong Kong surface waters and sediments

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The occurrence of pharmaceuticals in the natural environment due to extensive human use and release is well-documented. The widespread occurrence of these compounds in both abiotic and biotic samples has raised concern about their potential toxic effects with regard to both ecological and human health, especially regarding the increasing global prevalence of drug-resistant bacterial strains. We reported previously that wastewater treatment plants (WWTPs) in Hong Kong release high concentrations of some antibiotics into the local marine environment; likewise, clinical studies have shown that the rate of antibiotic resistance among some bacterial strains is very high in the city. This study measured pharmaceutical concentrations and bacterial counts/prevalence in seawater and sediment samples collected in Victoria Harbor, into which many WWTPs discharge their effluents, as well as more open marine waters and an aquaculture zone. Preliminary data show that 11 antibiotics were detected at the sampled locations, with azithromycin, cefuroxime and erythromycin-H_2O detected in all of the seawater samples at concentrations ranging from 5.6-181.6 ng/L. Lincomycin was only detected in Victoria Harbor samples, while clarithromycin was detected both in Victoria Harbor and the aquaculture zone, but not in more open marine waters. Preliminary bacterial analysis of the seawater samples indicated high total bacterial counts in the majority of the samples, while selective culture of Gram-negative rod-shaped bacteria isolated from the seawater samples indicated the presence of several resistant bacterial species, including potentially pathogenic *Vibrio* and *Aeromonas* species. Ampicillin-resistant species were found in all of the seawater samples, and species resistance to chloramphenicol and tetracycline were also present. Total bacterial counts in sediments were approximately 10-fold lower compared to those in seawater samples. Ampicillin- and tetracycline-resistant species were also identified in the sediments after selective culture was performed, though not all of the samples contained resistant bacteria, and resistant species were generally less prevalent in the sediment samples compared to the seawater samples. The results of these analyses will be used to carry out a probabilistic risk assessment of pharmaceuticals in the Hong Kong environment to determine their potential ecological risks.

MO059

Population level effects of embryo development disorders in the benthic key species Monoporeia affinis

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Embryo development in the Baltic Sea key species *Monoporeia affinis* has been used for decades as an indicator of environmental health in regional assessments and in the Swedish national monitoring program. Several laboratory and field studies have shown that this indicator can be successfully applied to monitor and assess effects of chemical substances as well as other environmental stressors, such as decreased oxygen levels and increased water temperature. Currently, embryo development in *M. affinis* is put forward as a potential core set indicator for chemical pollution assessment and monitoring in the Baltic Sea under the European Commission's Marine Strategy Framework Directive. Despite this indicator´s extensive use in environmental monitoring and assessments it is currently not known whether developmental effects translates in to effects at higher levels of biological organization. In this study we have developed matrix models that describe several populations of *M. affinis*, both in the Baltic Sea and in large lakes of Sweden. We use these models to estimate potential long-term effects on population densities due to increased rates of embryonic development disorders in *M. affinis*. Our results show that long term abundances could be drastically reduced at levels of disturbed embryonic development that have been observed at multiple sites along the Baltic Sea coast. Furthermore, we will present results, based on these models, which assess potential population effects of different future scenarios of increased chemical pollution, eutrophication and temperature.

MO060

Sediment associated-toxicity in the Mar Menor lagoon (SE Spain) under calm and stormy conditions: applicability of the sea-urchin embryotoxicity test

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Toxicity of surface sediments from the Mar Menor Lagoon (SE, Spain) was assessed by using the sea-urchin embryotoxicity test (SET). The bioassay was performed exposing fecundated eggs of *Paracentrotus lividus* to whole sediment and sediment elutriates, simulating calm and stormy conditions respectively. Results clearly indicated that storm/windy events may substantially increase the water toxicity associated with sediment resuspension through the water column in the Mar Menor Lagoon.

MO061

Baltic Sea sediments in a comprehensive ecotoxicological investigation using chemical analysis and a bioassay battery

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The Baltic Sea, one of the largest brackish water bodies, is considered to be an exceptionally sensitive and endangered marine ecosystem. The average residence time of Baltic Sea water ranges between 25 and 50 years, thus facilitating the accumulation of pollutants in sediment. From thirteen sites in the Baltic Sea sediment cores were collected and separated in different layers. For a ecotoxicological risk assessment chemical analysis and as set of bioassays were performed. Ecologically relevant processes such as bioconcentration and baseline toxicity of hydrophobic organic carbons (HOCs) are mostly controlled by the chemical activity (a) of the substance rather than by its total concentration in the sediment. To calculate chemical activity and thus to predict bioconcentration and baseline toxic potential of the contaminant mixture the freely dissolved concentrations (C_{free}) of PAHs and PCBs were measured in sediment pore water using solid phase microextraction, a passive sampling technique based on the principle of equilibrium partitioning. Sediment toxicity was assessed by comparing $\sum a$ to the range of a known to produce baseline toxicity. Extracts from PLE (pressurized liquid extration) and mild methanol/water extraction, were investigated for their dioxin-like potential (EROD-assay with the RTL-W1 cell line) and their embryotoxic impact on *Danio rerio* (Fish embryo toxicity test – *FET*). A sediment contact test (SCT) with *Danio rerio* using native sediments was performed for comparison. The mild methanol/water extracts revealed no effects in this study. In contrast the PLE extracts gave strong embryotoxic potential and indicated dioxin-like activity for all investigated sampling sites. Interestingly, PLE extracts in the FET showed similar results as the SCT using native sediments. Chemical analysis of PLE extracts was carried out to calculate ChemTEQ values. The results were compared to the BioTEQ values from the EROD-assay. ChemTEQ values up to 24 % of the RTL-W1 BioTEQ values were found. Results showed that despite elevated total PAH and PCB concentrations in Baltic Sea sediments, HOC bioavailability was limited since the estimated $\sum a$ was well below baseline toxicity levels. However, Baltic Sea sediments have a toxic potential as the bioassays showed. In addition to the baseline toxicity the bioassays reflect very specific toxicities. Furthermore the methanol/water extraction might not be useful for representing bioavailable HOCs.

MO062

Comparison of trace elements bioavailability and their bioaccumulation in Manila clam Venerupis philippinarum from Atlantic and Mediterranean estuarine environments.

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The concentrations of trace elements in aquatic environments may be enhanced in seafood, posing risks for human consumption. Among seafood products, marine bivalves play an important role in terms of exploitation and production. The Manila clam *Venerupis philippinarum* (Adams and Reeve, 1850) represents almost 20% of molluscs production worldwide, being Europe the second producer after China. In Europe, the species was introduced in many coastal systems, like Ria de Aveiro (Portugal) and Venice Lagoon (Italy), due to its high reproductive and growth rates and its tolerance to a wide range of environmental conditions. Health risks for human consumption can occur in these high harvested estuarine environments, since illegal fishing and collection of clams are occurring over regulated activities. In Venice Lagoon, the highly polluted industrial zone of Porto Marghera represents a dangerous site; in Aveiro Lagoon, the main toxicological concern is related to the Laranjo basin, an Hg highly polluted area prohibited to fisheries activities. Total metals and metalloids concentration is widely used as standard for food safety, although bioavailability of trace elements in the biota depends on the partition of elements into several geochemical phases of the sediments. The distribution in the different phases depends specifically on parameters such as pH, redox conditions, temperature, occurrence of organic matter and benthic activity. Thus, total element determination does not always yield sufficient information for an accurate risk assessment. As a consequence, speciation data are required to get knowledge about mobility, bioavailability and impact of elements on biological organisms. The aim of this study is to compare the data obtained for total metal content, bioavailability of trace elements in sediments of harvested areas, and bioaccumulation of trace elements in *V. philippinarum* in two different estuarine areas. In particular, the partitioning of trace elements by geochemical speciation was investigated to check for any similarity in behavior of trace elements depending on environmental conditions, and to understand the mechanisms of trace elements bioaccumulation in clams coming from two different harvesting regions. These data are fundamental to assess the risk for human consumption of clams, both for Aveiro and Venice coastal communities.

MO063

Monitoring toxicity of PAHs in intertidal sediments for five years after the Hebei Spirit oil spill in Taean, Korea

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Ecotoxicological monitoring of intertidal sediments was performed for 5 years after the *Hebei Spirit* oil spill in Taean, Korea. Sediment toxicity was observed on most of the beaches 4 months after the spill and later decreased rapidly to nontoxic levels 8 months after the spill. The concentrations of total polycyclic aromatic hydrocarbons (TPAHs) in the sediments ranged from 2 to 530,000 ng/g during the monitoring. More than half of the samples containing residual oils within sediment layers exhibited significant toxicity 5 years after the *Hebei Spirit* oil spill. Using a logistic regression model, the median lethal concentration of TPAHs to amphipod *Monocorophium uenoi* was estimated to be 36,000 ng/g. From the 63 chemistry and toxicity data, the effect range low, effect range median, threshold effect level, and probable effect level were derived to be 3,190, 54,100, 2,480, and 29,000 ng/g, respectively. The relative compositions of the PAH groups indicated that the weathering process is still ongoing.

MO064

Organic priority substances and microbial processes in marine coastal sediments (Adriatic Sea, Italy)

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PERSEUS EU FP7 Project (Policy-oriented marine Environmental research in the Southern European Seas) aims to identify the interacting patterns of natural and human-derived pressures on the Mediterranean and Black Seas, to assess their impact on marine ecosystems and, using the objectives and principles of the Marine Strategy Framework Directive as a vehicle, to design an effective and innovative research governance framework based on sound scientific knowledge. In the frame of this Project (subtask 1.3.3 ADREX: Adriatic and Ionian Seas Experiment), a preliminary monitoring survey has been conducted in the Adriatic Sea (Italy) in order to verify the occurrence and the variation of selected classes of organic priority substances in sediments and to study the structural and functional characteristics of native bacterial communities. The study site represented a good natural laboratory sensitive to climate variability and human pressure, owing to the semi-enclosed nature of the Adriatic Sea and to the increasing trend of human activities in the coastal regions. During the cruise ADRI 13, (November 2013) three coastal areas sited in front of Ancona, Gargano Promontory and Bari were sampled. In every sites surface sediment was collected and in selected sites dated sediment cores were analysed. The compounds investigated are included in the list of organic prioritary substances: PAHs, bisphenol A (BPA), alkylphenols (Aps), selected on the basis of the anthropogenic pressure. The extraction-clean-up was performed by ultrasonic bath with the appropriate solvent followed by analytical determination with LC-MS and HPLC UV-fluorescence. The microbiological analysis of bacterial abundance was determined by epifluorescence microscopy and flow cytometry; the rate of bacterial carbon production was determined by measuring the ^{3}H-leucine uptake rate and the community respiration was estimated by the measurement of the *electron transport system* (ETS) activity. Microbes associated with marine sediments play an important role in the C-flux being responsible for the transformation of organic carbon (autochthonous and allochtonous) into biomass. The results of these studies will improve the knowledge on how the environmental factors and the human pressure influence the changes in the assimilation or mineralization rates, affect the C-flux in the trophic chain and potentially the fate of organic pollutants.

MO065

Toxicity tests to assess the possibility to use clam harvesting waste as gabions filler material in the Venice lagoon

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Since *Venerupis philippinarum* introduction in 1983, clams harvesting has spread enormously in the Venice lagoon. This kind of activity include an operational phase that consists in sieving all material previously collected, picking out clams of commercial interest. During this phase operators obtain a waste that they usually discard in lagoon. This kind of waste, that is mostly composed by shells, wood, sediment and different kind of inert materials, can accumulate in specific areas in the long term. The accumulation of this material on the seabed, particularly in Manila clam (*V. philippinarum*) fishing concessions, can interfere with fishing operations by reducing the collection efficiency. According to Institutions responsible of fisheries resources supervision in the Venice lagoon, the management of this waste has become an urgency. So we worked to define a strategy in order to collect this material and to avoid the need for its disposal; finally we took into account the possibility to use it as a filler, in total or partial replacement of the material currently used, for manufactured devices such as gabions and submerged barriers to be used for banks protection and consolidation in the Venice lagoon as well as for beaches protection from erosion. In order to deepen the knowledge of this kind of material, potentially usable for engineering works aimed at morphological and environmental protection and to establish if it can be considered safe and stable, we planned a series of ecotoxicological experiments. Toxicity tests were carried out on a matrix composed of environmental waste material mainly made by clams resulting from the sieving phase and collected during a sampling campaign in the Venice lagoon. It was necessary to investigate the presence of any toxic effects, due to release phenomena and synergies, through the implementation of three batteries of ecotoxicological tests with three different species (*Vibrio fisheri*, *Mytilus galloprovincialis* and *Phaeodactylum tricornutum*). We evaluated the toxicity of elutriates obtained from homogeneous matrix representing wastes obtained from *V. Philippinarum* fishing in different collection areas in the Venice lagoon. This work provided the opportunity to better define all characteristics of this material that can't be derived from simple multielemental chemical analysis: particularly we could predict his behavior once reinserted into the aquatic compartment inside gabions or submerged barriers.

MO066

Toxicity test method based on the inhibition of reproduction in Ulva: revision

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During progression of reproduction the green macroalga, Ulva, shows a visible change in thallus colour from yellow green (vegetative stage) to dark olive (reproductive stage) and then to white at the terminal stage of reproduction. The extent of reproduction is determined by image analysis measurements, and/or visual inspection of the proportion of the reproductive area out of the total area. Based on the inhibition of reproduction in Ulva, a new bioassay method was developed. Setting up the *Ulva* bioassay is quite easy and the test is simple to follow. However, the visual quantitative scoring of the discoloration of the disks was not easy and was often subjective since the exact "vegetative" vs "reproductive" parts are sometimes hard to differentiate although a difference between the treatments and controls can be clearly seen. We have therefore conducted a wide range of experiments using various measurement techniques including:a) Spectrophotometric measurements of *in vivo* thallus and photosynthetic pigments (after solvent extractions), b) fluorometric measurements of *in vivo* thallus and photosynthetic pigments (after solvent extractions), c) Staining of reproduced thallus portions. d) Green color recognition image analysis. So far, we have found that the use of staining method using Evans blue and the use of image J programme is worth exploring further as it would complement the present methodology. by providing more objective criteria for discerning the color change due to reproduction.

MO067

Parhyale hawaiensis as an alternative organism in marine toxicity tests

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With the increase of urbanization and pollution of coastal areas around the world, there is a concern with the preservation of marine environment. Therefore representative organisms of this environment, which will serve as a source of data for the regulatory field and protection of aquatic life are important. *P. hawaiensis* is an marine amphipod that occurs in the American coast and it is easy to grow in laboratory conditions. The aim of this study was to verify if *Parhyale hawaiensis* could be an alternative organism in toxicity testing. A basic protocol for culturing and testing was elaborated, testing was performed with a reference substance (Zinc Sulphate) and the developed protocol was applied to test two textile azo dyes CI Disperse Red 1 and CI Disperse Red 13. The average LC50 (96h) for zinc was 0.60 mg $Zn.L^{-1}$ (±0.30, n=9). Tests showed good repeatability and sensitivity to Zn was similar to the response with other amphipods. Disperse Red 13 presented an average LC50 (96h) of 1.94 $mg.L^{-1}$ (±0.94, n=3). LC50 (96h) for Disperse Red 1 varied from 3.1 to 37.4 $mg.L^{-1}$ (n=4). Precipitation of the Disperse Red 1 was the probable cause of the highly variable LC50. The results of this study suggest that *Parhyale hawaiensis* is a promising organism for ecotoxicological studies. More experiments are being conducted for optimization of culturing and testing conditions. **Acknowledgments:** FAPESP Thematic Project 2008/10449-7, Scientific Innitiation fellowship 2012/09512-1 for AS; Master fellowship 2010/14033-0 for MCA.

MO068

Optical detection of low concentrations of toxic nano particles in saline solutions; approximating a pseudo-estuary environment

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One of the holy grails of environmental toxicology is the detection of very low concentrations of toxic nanoparticles in the aquatic environment. In the laboratory there are established methods that enable these measurements to be made. SEM can be used with a sample where the liquid has been evaporated and this can reveal size, shape and chemical composition. Dynamic light scattering or laser diffraction are also established methods of particle characterisation;providing both size and shape information. SPR is label free detection, but particles can be identified as the SPR angle is directlty determined by the particles refractive index. In this paper we explore the detection of Ag nanoparticles and silver nitrate in de-ionised water and saline solution. The detection of the SPR phase response is also measured, to explore extended detection sensititivty.

MO069

Physical stressor (temperature tolerance) test exposition with temperate sea anemone Actinia equina, a climatic and environmental changes simulation

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Atlantic and Mediterranean warming-related diseases outbreaks and species shifts have recently been documented. Biomarkers of short-term effects on the health or resistance of organisms are necessary to assess and understand mechanisms affecting marine biodiversity. Until now, climate warming has been studied at population or community level. Here we offer a better understanding of such phenomena at the organism level, using anatomic-morphological approaches to interpret effects of natural physical stressors, according to behavioral patterns. Elevated temperature and solar radiation, are now recognized as the primary environmental stresses that lead to mass benthonic organisms, mainly cnidarian bleaching. Intertidal organisms are subject to a variety of stresses such as desiccation, water temperature, acidification, increase salinity, nutrient limitation, space competition and predation. The thermal stress is a knower such a mainly environmental factor responsible for climatic and environmental changes. This study takes a behavioral (morphological and anatomic parameters) to identifying changes soon after exposure to physical stressors in the temperate sea anemone *Actinia equina*. Sea anemones were subjected to variation temperature range over a 96 h period. Behavior endpoints were divided to be differentially showed as a function of temperature stress. Behavioral patterns analysis placed the differentially ecological functions in a wide range of categories including tentacle flexion, tentacle retraction, column cavitation, peristome depression and oral disc flexion. This suggests that the early stress response to elevated temperature involve essentially all aspects of same chemical reactions, in this case we observed an receptors functioning and the frequency of open-close oral sea anemones, tentacles and columns anatomic alterations to detect earlier the effects of physical stress induction.

MO070

Characterization of an ecdysone receptor and a vitellogenin-like cDNA in the estuarine copepod eurytemora affinis

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Aquatic ecosystems constitute the chemicals' final destination. Since 2000, European Union acts to protect them through the Water Framework Directive. One of the chemicals' families targeted by this directive is pesticide. Their endocrine disruption (ED) potential is well documented in vertebrates. However, few data are available on invertebrates whereas they represent 95% of the wild fauna. The development of specific tools to diagnose ED exposure and/or effects in invertebrates is then required. Among candidate biomarkers, the induction of the female-specific vitellogenin (VTG) protein in males is standardly used, in particular in fish. Moreover, in arthropods, sexual maturation and molting are controlled by the hormone ecdysone which acts through its cognate receptor, the ecdysone receptor (EcR). In this context, the present study consists in developing Vtg and EcR assays in the sentinel crustacean *Eurytemora affinis*. This widespread calanoid copepod is considered as a key species in the food-web of temperate estuaries. We isolated both Vtg and EcR cDNAs in *E. affinis* using degenerated and RACE-PCRs. Phylogenetic analysis of Vtg revealed that the identified gene is an ortholog of the *vtg2* gene. The VTG shares a low amino acid sequence identity with copepods (20%) and eumalacostracans (< 10%) whereas the EcR was moderately conserved in both subclasses (40%). The mRNA basal levels and fluctuations associated with life cycle were assessed by quantitative PCR in both males and females. Finally, in order to evaluate the relevance of the use of Vtg and EcR transcript quantifications as ED biomarkers, mRNA variations will be evaluated after exposure to model endocrine disruptors.

MO071

Development of a humpback whale cell line as a versatile tool for in-vitro toxicity assessment

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Persistent Organic Pollutants (POPs) are widely produced chemicals characterized by their persistence, bioaccumulation, toxicity and long-range environmental transport. Semi-volatile POPs are transported by air over long distances and are detectable in Antarctic biota. Polar foraging humpback whales *(Megaptera novaeangliae)* are dependent on a lipid-rich diet and accumulated lipid stores to undertake the longest migration and associated period of voluntary fasting, known in any mammal. This extreme life-history leads to an elevated sensitivity to the effects of POP toxicity. Measuring the toxicological impact of POPs on wild populations of humpbacks is a greater challenge in chemical risk assessment. Herein, we report for the first time the successful derivation of humpback whale cell cultures gained from integument tissue. Whales were biopsied late in their migration with flotation darts fired from an air rifle. Biopsies were collected from the dorsal area and measured 0.8 cm x 3.5 cm. Separated tissue was processed and maintained at 37°C, 5% CO_2 and in sterile growth medium. After 10 days the first cells grew out of tissue explants and high proliferation was observed after 20-25 days. The interface tissue lying between the epidermis and blubber section has shown to be most suitable. Obtained primary cells showed fibroblastic morphology with the typical fibrous and long-spindled form. Infinite long-term cell cultures will be obtained by mechanically induced mutations delivered by using a plasmid encoding the simian virus (SV 40) large T antigen. Early passaged and immortal cell cultures will be characterized according to morphology, growth, cryogenic preservation, metabolic activity, karyotype variations and fibroblast specific markers. This project aims to assess the toxicological impact of identified priority POPs in whale blubber by multiple- endpoint cytotoxicity assays. Humpback whale blubber and Antarctic krill extracts will serve as a novel exposure medium mimicking environmentally relevant mixtures representative of the main diet of adult and nursing young. The derived cell line will contribute a unique tool to study the effect of organic pollutants and provide more accurate and reproducible approaches for integrated toxicity risk assessment of humpback whales.

MO072

Biochemical responses induced by sucralose in Daphnia magna

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Sucralose is an intensively sweet food additive derived from sucrose in a process where three hydroxyl groups are substituted for three chlorine atoms, producing a very stable substance and has become a popular substitute for sugar. Its exceptional stability in combination with high water solubility and popularity has resulted in measurable concentrations not only in recipient waters but also further out in the oceans. Animal and human studies have concluded that sucralose is safe for human use and earlier studies in aquatic organisms indicate low bioaccumulation potential and negligible acute/chronic toxicity. However, in non-standardized surveys, significant feeding and behavioural alterations have been reported in crustaceans, indicating possible sublethal behavioural effects. We hypothesized that these effects are related to alterations in acetylcholinesterase (AChE) and oxidative status and investigated changes in AChE and oxidative biomarkers (oxygen radical absorbing capacity, ORAC, and lipid peroxidation as thiobarbituric acid reactive substances, TBARS) in the crustacean *Daphnia magna* exposed to sucralose (0.0001-5 mg L^{-1} of). The results showed that the sucralose concentration was a significant positive predictor for ORAC, TBARS and AChE activities in the daphnids, although the biomarker responses followed non-monotonic concentration-dependent relationships. These responses support our hypothesis and suggest that exposure to sucralose may induce neurological and oxidative mechanisms that might have consequences for animal behaviour and physiology.

MO073

Filling the gaps in our knowledge of the effects of priority Hazardous and Noxious Substances on marine biota.

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Hazardous and Noxious Substance (HNS) can be defined as any substance other than oil, which if introduced into the marine environment is likely to harm living resources and other marine life. There is a current paucity of knowledge about the effects of HNS on marine biota. Effort in filling information gaps should be focussed on priority HNS. Priority HNS can be selected on the basis of their known toxicity and carcinogenity, Bioaccumulation, Biodegradation, physico-chemical properties, incidence of previous spills and the likelihood of future spillage at sea. On this basis aniline, butyl acrylate, hexane and trichloroethylene were selected as priority chemicals to gather toxicity data Four marine species, *Tisbe batagliai, Pomatoceros triqueter, Fucus vesiculosus* and *Ceramium tenuicorne* were tested across a range of concentrations of these four priority HNS, test endpoints were mortality, embryonic development, growth and germination plus growth respectively. Tests were carried out in well plates and included solution renewal at regular intervals. Average LC50's (n=3) for *Tisbe* were 3.83 and 7.48mg/l for aniline and butyl acrylate respectively. *Pomatoceros* $EC_{50's}$ were 34.4 and 10.6mg/l for aniline and butyl acrylate. Hexane did not produce an effect for *Tisbe* but an EC_{50} of 172mg/l was calculated for *Pomatoceros*. Trichloroethylene did not produce an effect on either animal species. *Ceramium* showed an EC_{50} of 2.01mg/l for aniline whilst the three remaining chemicals elicited a positive growth response. *Fucus vesiculosus* was not affected by any of the chemicals at the concentrations tested. The different levels of effect between chemicals reflect their physico-chemical properties, hexane and trichloroethylene have the highest vapour pressures of the chemicals tested and so are likely to be lost from tests solutions more readily, this is reflective of the dynamic exposure conditions during a marine spill. Aniline is the most soluble of the chemicals tested and so produced the greatest effect. To the authors knowledge this is the first toxicological data for butyl acrylate on marine species, and the first results for any of the chemicals tested on marine algae species. These results can be used to develop post spill risk assessments for these chemicals. *This work has been carried out with support from Defra, Project CHIME E5204 and in the frame of ARCOPOLplus project, co-funded with ERDF through the Atlantic Area Transnational Programme*

Periphyton as bioindicator and community model – critical review, work in progress, future perspectives (P)

MO074

Development of a tiered approach for the characterization of periphyton on the genetic and functional level

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Periphyton is a taxonomically diverse and dynamic community of bacteria, algae, and fungi that provide essential ecosystem services to streams. The sensitivity of periphyton to environmental conditions including anthropogenic stressors makes it an important bioindicator. However, the complexity and dynamics poses a challenge to identifying appropriate descriptors, a useful level of detail and linking observed changes to understand underlying mechanisms. Against this background we aim to establish a tiered assessment of periphyton status using flow cytometry as first-tier followed by more detailed analysis of diversity and structure, as genetic diversity (community fingerprinting by Automated Ribosomal Intergenic Spacer Analysis [ARISA]), 3D structure (by Confocal laser scanning microscopy [CLSM]), and extracellular polymeric substances (EPS). Flow cytometry and corresponding data analysis are being adapted to the characteristics of periphyton to analyze changes of functional groups (i.e. organism size, growth form, pigmentation) representing biological characteristics that are connected to ecosystem functions. This data will be linked with information on the genetic diversity and taxonomic species composition as well as structure and extracellular chemistry and integrative endpoints such as biomass and photosynthetic activity. These suggested tiered approach to periphyton characterization will finally be applied to asses effects of the herbicide Terbuthylazin (TBA) and insecticide Imidacloprid (IMI), that in preliminary experiments have shown to affect periphyton. To this end, we have established an indoor colonization setup for long-term culturing of periphyton and exposure to chemicals and are in the process of the molecular diversity analysis, whereas we currently are developing primers specific for green algae, diatoms and cyanobacteria. Keyword: Periphyton, First-tier approach, Flow cytometry

MO075

Approaching real complexity: toxic impact of a mixture of diuron and propranolol on periphyton.

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The efforts done in water resource management have allowed to reduce concentrations of priority pollutants in rivers; however an increasing number of chemicals can now be found at low concentrations in the aquatic environment. Where agricultural lands are next to urban areas, it is not rare to find known chemicals (herbicides, pesticides, etc...) in mixture with emerging pollutants (pharmaceuticals, personal care products or nanoparticules). To approach such a complexity it is essential to assess mixture toxicity on multi-species systems rather than assessing toxicity of single substance on single species. Within aquatic ecosystems, periphyton have been shown to be good indicators of toxicity and so represent a pertinent community model for ecotoxicology. From this perspective, acute toxicity of the herbicide diuron (photosynthesis inhibitor) and of the β-blocker propranolol (pharmaceutical product included in the list of emerging pollutants) towards periphyton has been assessed, both singles substances were tested as well as an equitoxic mixture. The effects observed on communities were also compared to effects observed on a single-specie population of the diatom *Cyclotella menegheniana* to highlight the pertinence and the power of the community approach. Indeed the community approach allowed revealing both direct and indirect effects of these 2 chemicals on periphyton. Indeed, photosynthetic efficiency was directly affected by diuron (EC_{50}=8.11 µg/L) but also by propranolol (EC_{50}= 3.1 mg/L). Moreover, the toxicity of these 2 chemicals in equitoxic mixture followed the Concentration Addition concept, indicating that even if propranolol is present at very low concentration in rivers, it would participate in the photosynthesis inhibition due to a mixture of diuron-propranolol. Each contaminant tested separately had no effet on bacterial activity (leucine-aminopeptidase extracellular enzyme activity) of periphyton nevertheless exposure to the mixture of diuron and propranolol provoked an increase in bacterial activity, reaching 140% of control activity at the highest concentration tested. This study illustrates the importance of the community approach to detect direct and indirect effects of toxic mixture on communities. Moreover, it highlights the

importance of mixture effects on ecosystems since even at low concentrations emerging pollutants can participate to the overall toxicity.

MO076

Experimental study of arsenic detoxification by periphyton

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Arsenic pollution in water has an important impact in human and ecosystem health. In this experiment, arsenic detoxification was studied using periphyton or fluvial biofilm, an indicator of the ecological status of aquatic ecosystems, and the influence of the fish *Gambusia holbrooki*. These organisms were exposed for 13 days to 130 μg/L of arsenic (specifically As^V). The experiment comprised four treatments: control (fish), periphyton, arsenic, and periphyton with arsenic. Results showed a negative effect of arsenic on algal periphyton biomass and photosynthetic capacity. Arsenic also caused a stress effect in fish that resulted in increased ammonia excretion. Moreover, phosphate production via bacterial mineralization of fish faeces proved important. In conclusion, arsenic contributed to eutrophic conditions and impaired periphyton's purification capacity.

MO077

Influence of epipsammic biofilms on the retention and speciation of arsenic in freshwater environments

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Arsenic (As) is a highly toxic metalloid which causes severe health problems worldwide. In aquatic environments, As may undergo transformations in its chemical form due to its interaction with mineral surfaces, but also through (micro-)biologically mediated reactions (biotransformation), which can strongly affect its mobility, bioavailability and toxicity. In this work, the influence of biofilms developed onto riverbed sediments on the (bio-)adsorption and/or (bio-)uptake, mobility, and (bio-)transformation of As^V was studied in microcosm systems. The results obtained were linked with toxicity assessment. Biofilm enhanced the removal of As^V from the water up to 91% of its initial concentration, while the removal was only 63% in the sediment without biofilm, after 14 days exposure. The presence of equimolar P concentrations enhanced the amount and the rate of As removal in the systems with biofilm, but had not effect in absence of biofilm. Biofilms strongly changed the speciation of As in the water column. For the sediment/biofilm system, aqueous As was mostly (~98%) As^V, whilst As^{III} accounted for 1.2% of total aqueous As. MMA^V and DMA^V represented only the 0.6 and 0.7% of total As. In contrast, As^{III} accounted for 39% of aqueous As when biofilm was absent, with the remaining (61%) in the form As^V. Methylated aqueous species were negligible in absence of biofilm. The distribution of As in the biofilm showed that ~30% of the As retained was “extracellular As” (extracted by phosphate), most of it (>99.5%) in the form of As^V. In contrast, in the absence of biofilm, phosphate extracted up to the ~65% of sorbed As, ~12% of which was As^{III}. In comparison with sterilized sediments, biofilms decreased the release to water of previously taken As from 8.5% in absence of biofilm to 4.3%. Most of the released As was As^V (94%), with only 3% as As^{III}, when biofilm was present. In contrast, As^{III} accounted for 21% of the total released As in the systems without biofilm. It is concluded that biofilms covering riverbed sediments play a key role in the cycling of As in river environments. They not only promote the removal of As^V from the water and decrease the subsequent remobilization of As, but also strongly affect the speciation of As in the water column by inhibiting the occurrence of aqueous As^{III}. This fact has noteworthy toxicological and geochemical relevance, as for remediation purposes, considering the higher toxicity and mobility of As^{III} species.

MO078

Kinetics of arsenite removal and (bio)transformation by a natural multi-species biofilm growth on riverbed sediments

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Arsenite (As^{III}) is the form of As exhibiting the highest toxicity and mobility in natural media. Biofilms can play an important role taking up and biotransforming As to less toxic chemical forms. In this work, the uptake and biotransformation of As^{III} by a natural biofilm was studied at microcosms scale. Biofilm-rich sediment samples (~500 g wet weight, 35% wc) from the Anllóns River (NW Spain), which runs through an As-rich gold mining area, were incubated under controlled conditions. Cultivated biofilm was exposed to As^{III} (300 µg/L, 99%, 2.2 µgAs/L after 14 d) and fast (>95%, 8.0 µgAs/L, in 1 d). In these systems, As^V predominated in the water (65–95% of total As) at any time, whilst the fraction of aqueous As^{III} decreased from 34% after 2 h to 12% after 14 d exposure (271 ng As^{III}/L). The ratio As^V/As^{III} increased from ~2 (2 h) up to ~20 after 8 d exposure, suggesting oxidation of As^{III} took place. The concentration of DMA^V increased up to 615 ng/L after 14 d, suggesting detoxification by biomethylation also ocurred in the biofilm. Volatile As forms accounted for < 1% of removed As. In contrast, in the absence of biofilm the concentration of aqueous As was much higher (10–40 fold) and the removal kinetics was slower than in presence of biofilm. Besides, the ratio As^V/As^{III} increased with time from ~0.3 to ~2. As^{III} predominated (~75%) within 7d exposure, then As^V predominated onwards (65% at 14d). The concentration of As^{III} decreased with time from 63 (2 h) to 7 µg/L (14 days), whilst the As^V concentration was roughly constant at 15±4 µg/L. DMA^V was also found in these systems only after 8 d of incubation (563 ng/L). Biofilms growing on riverbed sediments not only rapidly take up soluble As^{III}, but they also greatly inhibit its occurrence in the water column; conversely this As form predominates when As^{III} is added to sediments devoided of biofilm. This presumably would reduce As toxicity and mobility, as make easier remediation actions.

MO079

Extracellular enzymatic activity of intact heterotrophic biofilms is decreased upon exposure to TiO2 nanoparticles and environmentally realistic UV radiation

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The growing use of TiO_2 nanoparticles (TiO_2 NPs) will inevitably result in an increased environmental release of these materials, which may pose a risk to aquatic communities, such as heterotrophic freshwater biofilms. Depending on the water chemistry, TiO_2 NPs entering aquatic environments are prone to agglomeration and sedimentation. These processes result in increased exposure of biofilms to TiO_2 NPs, with thus far little knowledge about potential consequences. Since TiO_2 NPs are highly photoactive and generate reactive oxygen species (ROS) upon irradiation, one possible mechanism by which TiO_2 NPs may affect biofilm function is through ROS damaging components of the biofilm. As part of the biofilm matrix, extracellular enzymes might represent a first site of encounter for ROS produced by TiO_2 NPs.\nIn this work we investigate, if the extracellular activity of three enzymes essential for nutrient cycling in intact heterotrophic biofilms is decreased by simultaneous exposure to TiO_2 NPs and environmentally realistic UV intensities. Exposures were conducted with intact heterotrophic biofilms and TiO_2 NPs coated with different substances, including environmentally (rutin, tannic acid) and biologically relevant coatings (dopamine, phenylalanine, ascorbic acid) and more general coatings such as acidic and basic, nonpolar organic and molecules with different chromophores. These coatings mimic the variety of engineered and naturally occurring surface modifications that may be present for TiO_2 NPs and allow for the determination of the mechanism by which particle behavior is affected.\nExposure to UV and TiO_2 NPs coated with catechol and alizarin red and P25 decreased activity of the extracellular enzyme β – glucosidase (carbon – cycling). This was linked to the efficient production of ROS by these particles, with P25 being the most active. L – leucin aminopeptidase (nitrogen – cycling) activity was attenuated only by P25 exposure and the alkaline phosphatase (phosphorus – cycling) did not decrease following exposure to any of the TiO_2 NP and UV combinations. Comparing the impact on extracellular enzymes in intact biofilms with the effects observed for an isolated enzyme furthermore demonstrated that extracellular enzymes within the biofilm are better protected against ROS than enzymes present freely in solution. Overall, the significant decrease in activity may adversely affect nutrient acquisition by the biofilm and nutrient cycling in freshwater environments.

MO080

Ecotoxicology of polycyclic aromatic hydrocarbons (PAHs) at the eukaryote-prokaryote boundary: eukaryotic zoospores and PAH-degrading bacteria

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This contribution focuses on the ecotoxicological impacts of PAHs in microbial communities formed by oomycetes and PAH-degrading bacteria. Here, we aim to evaluate the production, chemotaxis and settlement of eukaryotic zoospores produced by a rhizosphere oomycete, *Pythium aphanidermatum*, under a variety of ecological scenarios that mimicked the pollution by PAHs, including an active biodegradation by the bacteria. Among a common set of PAHs, phenanthrene and naphthalene revealed the strongest negative effect on the induction and production of the zoospores. The co-existence with PAH-degrading bacteria allowed zoospore production in these PAH-polluted scenarios. Zoospore chemotaxis was tested with selected environmental matrices (humic acids, organic solvents and root exudates). An attraction to these matrices occurred, or even it was enhanced, in the presence of PAHs (naphthalene and phenanthrene) and PAH-degrading bacteria. The zoospore settlement on the surface of liquid hydrocarbons (hexadecane and heptamethylnonane) occurred through selective sensing by the zoospores, which colonized hexadecane but not the methyl-branched hydrocarbon. Co-settlement with bacteria was only observed on hexadecane. We propose that the quantitative assessment of production and chemotaxis of the zoospores could be developed as a model to evaluate the environmental risk of PAHs. Furthermore, the community of both zoospores and bacteria seemed to provide a unique commensalism lifestyle in PAH-polluted ecosystem, which could be a strategy for further development of co-culture approaches in bioremediation of PAHs.

MO081

Development and validation of advanced monitoring systems for waterborne organic priority pollutants using microalgae biosensors

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The Water Framework Directive 2000/60/EC requires that Member States shall ensure the establishment and/or implementation of emission controls based on best available techniques. So far, the time-to-analysis required in commonly used monitoring methods determine a slow and in some cases ineffective response to detect accidental spills. In this context, it seems clear that cost-effective technologies and methodologies for real-time and average concentration monitoring will be necessary. For the real time detection a prototype has been developed with two main components a Filtration-Concentration-Clean Up unit (FCCU), which is devoted to concentrate the water sample up to 100 times using the principle of solid phase extraction, improving in this way the sensitivity; and the optical biosensor that contains selected microalgae immobilized into porous silicone (biomembrane) as the recognition element, and an O2-sensitive luminescent thin film as transducer of the photosynthetic status. A fiber-optic optoelectronic device, based on emission phase-shift measurements, interrogates the O_2 transducer that is placed in close contact with the biomembrane. The biosensor comprises a dual sensing head (Patent PCT/ES2008/000465). One of them contains a sensitive strain and the other a resistant one. To measure the average concentration a continuous flow integrative sampler (CFIS) was used. It consists of a peristaltic pump that flows the sample alternately through a sorbent material that retains the dissolved pollutants and a particle filter that retains the suspended particle, which facilitates the analysis of the priority pollutants in the dissolved fraction. Experiments were performed in a wastewater treatment plant located in the metropolitan area or Barcelona (Spain) where both devices were installed for half year. The monitoring system has been validated for quantification of seven target priority pollutants. Validations of both devices were carried out by determination of quality parameters such as detection limit, selectivity, response time, precision, accuracy, repeatability and reproducibility. Results show that those are valuable tools to quantification of the presence of priority pollutants in effluents. AKNOWLEDGEMENTS This work was supported by the LIFE+ Programme of the European Commission through AQUATIK project (LIFE 10 ENV/ES/000521). Authors acknowledge Prof. E. Costas for the work done in the selection and growth of microalgae used in biosensor.

MO082

Effect of temperature on the toxicity of mercury in different periphyton communities

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The present study describes the research conducted about the effect of increasing temperature on the toxicity of mercury on 3 different periphyton communities, which were obtained through natural colonization of substrates positioned in 3 ecosystems of Gallego river basin (NE Spain): High Mountain, chemical pollution area and agricultural pollution area. Obtained communities were transported to the laboratory for a microcosm experiment, where an acute toxicity test was performed. A structure was built with 12 methacrylate channels connected by hoses to the containers with different contents of mercury concentrations, all of them submerged in a tank with circulating water to the desired experimental temperature: each site actual average temperature and +5ºC. In order to quantify the biofilm photosynthetic efficiency according to each toxic concentration, fluorescence emitted by chl-a biofilm was measured with Mini-PAM (Pulse Amplitude Modulated) Fluorometer. These measures were used to estimate the effective concentration that reduces the photon yield by 50 % (EC_{50}). Results show an increase in toxicity of the compounds in all communities when increasing temperature. Furthermore, communities affected by chemical contaminants have greater resistance to the toxic than the other two communities, at both the current temperature and at 5 ° C more, which could be due to Pollution-induced community tolerance (PICT).

MO083

Responses of diatoms exposed to a fungicide and a petroleum distillate in stream mesocosms: sensitivity of trait-based metrics and community indices

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Diatom communities are widely used in biomonitoring of the quality of aquatic environments through a variety of biological indicators. Nevertheless, community responses have poorly been used in Ecological Risk Assessment of chemicals at higher tier studies (*i.e.*, mesocosms). Recent studies have encouraged the definition and use of diatom biological and ecological trait-based approaches which are supposed to be more integrative of environmental changes. In this study, traits defined in the literature were tested using classification into three ecological guilds (*i.e.* high profile, low profile and motile), identification of ecological traits of individual taxa (*i.e.* substrate relationships, mobility…), occurrence of life-form traits (colony, no-colony, fixed, mobile), and distribution into taxa size-class traits (5 size-classes). Moreover, the structure of diatom communities was investigated through biomonitoring indices, such as DBI (Diatom Biological Index) and PSI (Pollutant Sensitivity Index), and taxonomic approaches. Outdoor flow-through mesocosms were used to assess the effects of chemicals on diatoms in realistic and complex experimental environment including abiotic and biotic interactions. They also allowed assessing the sensitivity of the various endpoints cited above during the treatment period and the ability of communities to recover from disturbance. Chemicals, thiram (dithiocarbamate fungicide) and a petroleum distillate, were chosen with respect to their specific modes of action. Loadings were 35 and 170 μg/L for thiram and 0.01, 0.4, 2 and 20 mg/L for petroleum distillate. All treatments were performed in duplicates and four systems were kept as the controls. Streams were continuously treated for 3 weeks, followed by a two month-long post-treatment period. Diatoms were sampled every three weeks, before, during and after treatment period. Our results showed no effect of exposure using biomonitoring indices whereas some traits and the taxonomic structure clearly highlighted effects. In particular, the use of ecological guild, size-class and life-form response allow grouping taxa according to their their ecological profiles, providing better overview of ecological changes due to chemical exposure and highlighting short- and long-term effects on the biofilm structure and functioning. This demonstrates higher sensitivity of diatom traits to chemical exposure, thus supporting their interest in ERA. Keywords : Abundances, long-term vs. short-term effect, recovery dynamics, different scales comparison.

MO084

Long-term effects of triclosan on marine periphyton communities in flow-through microcosms

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Triclosan is a broad spectrum antibacterial and antifungal agent, used in formulations as diverse as plastics in children’s toys, kitchenware, toothpaste, clothes, soaps, carpets and first aid products. Several studies have shown triclosan to be toxic to bacteria and fungi, but it has also been shown to be highly toxic to algae with multiple mechanisms of action. We have investigated the long-term effects of triclosan on marine periphyton communities in a flow-through microcosm study. Results show that triclosan induce community tolerance, detected as short-term inhibition of photosynthesis, according to the Pollution-Induced Community Tolerance (PICT) concept. PICT was accompanied by stimulated chlorophyll *a* content and incorporation of radiolabelled carbon at 100 nM. Whether these changes originate from increased biomass or increased chlorophyll *a* synthesis and photosynthesis is, however, unclear. At the same exposure level the pigment composition in these communities were altered. Although the tolerance mechanism(s) is not known, photosynthesis and pigment composition seem important for tolerance to triclosan in algae and cyanobacteria. In addition we studied multivariate catabolic capabilities of bacteria in control and exposed communities using Biolog EcoPlates. These data are currently being analysed and will also be presented on the poster.

MO085

Is the composition of fungal populations in surface waters an appropriate parameter for the risk assessment of multiple pesticide loads from fruit cultivation?

A. Talk, Helmholtz Zentrum Muenchen Deutsches Forschungszentrum; S. Kublik, Helmholtz Zentrum Munchen / Research Unit Environmental Genomics; R. Berghahn, Federal Environmental Agency / Field Station Marienfelde; S. Mohr, Umweltbundesamt / IV; M. Engel, M. Uksa, G. Welzl, M. Schloter, Helmholtz Zentrum Munchen / Research Unit Environmental Genomics
So-called 'application sequences' in apple cultivations consist of many pesticides, which are repeatedly applied in short time intervals one after the other. The risk assessment of pesticides, however, includes only the evaluation of single substances so far. Pesticide loads reach surface waters through several pathways such as e.g. spray drift and runoff. This raises the question, whether continuous contamination of surface waters with pesticides over time may lead to negative effects on aquatic ecosystems although the individual substances should not have effects on aquatic organisms. Aquatic fungi are important for surface water ecosystems because they supply energy through litter decomposition. In ecotoxicological risk assessments aquatic fungi are not yet included as standard organisms although 'application sequences' comprise especially fungicides. In a mesocosm study the effect of an 'application sequence' scenario on the diversity of aquatic fungi was examined through fingerprinting of fungal communities colonizing Alnus glutinosa leaves in order to find out if this is a good parameter for the risk assessment of pesticides. •In general fingerprinting is a promising tool to examine aquatic fungal communities since the temporal succession in fungal communities could be displayed. •However, fingerprinting alone may not be sensitive enough for the detection of minor effects originating from multiple pesticide loads. •Sequencing may allow for a deeper look into the fungal communities.

Waste and Wastewater effluents: chemical and ecotoxicological characterisation (P)

MO086
In situ GamTox test to assess toxic pulses and monitor water quality
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Gammarus spp. are widely spread pollution indicators in brooks and streams. GamTox represents a simple field test to assess and monitor toxic stress caused by both point and diffuse pollution sources. The test has been applied in different case studies and pollution scenarios, such as three WWTPs, one horticulture and one wood processing industry. The test has been performed at each site as follows: 5 replicate plexiglass tubes sealed on both ends with nylon net caps (0.5 mm mesh size) containing each 10 parasite-free medium-sized gammarids and one conditioned alder leaf were placed in a basket and fixed on the streambed and at the banks. Survival and feeding rate (% leaf loss) were monitored on a weekly basis for a duration up to 6 weeks. Test animals were caught in the same catchment at unpolluted head stream sites. Gammarids responded within two weeks of exposure to pesticide/neurotoxic insecticide pollution and pharmaceuticals from WWTPs. No responses could be recorded as effects of a wood processing industry (Fenoxycarb). We discuss the application of GamTox in routine biomonitoring as screening and diagnostic tools, as well as the limits of the method regarding e.g. stream type, reliability of feeding as test parameter, etc.

MO087
Monitoring and diagnosis of pollution peaks in waste water treatment plants: concept and case study
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Urban waste water treatment plants face the challenge to operate optimal purification processes independent of varying concentrations and composition of substances in the incoming pollution cocktail. During a purification process of several hours the ecotoxic potential needs to be eliminated to protect aquatic biota in the receiving water bodies. Moreover, waste water and sludge will be submitted to re-use and re-cycling of nutrients and energy in the near future. To achieve these ambitious goals several measures are necessary to secure water and sludge quality: 1) monitoring of the inflow, 2) optimisation of the purification, 3) monitoring of the outflow. While chemical online monitoring has already become a routine tool, biological-ecotoxicological online monitoring starts to receive attention, too. Online biomonitoring with ecological relevant and pollution-sensitive running water bioindicators such as *Gammarus* spp. allow for the evaluation of both short-term responses and long-term effects of the waste water on the receiving ecosystem. In case of an alarm situation such as increased mortality a water sample can directly be taken for subsequent chemical diagnostics and additional ecotoxicological testing. This 3 step approach: biomonitoring- analytics- ecotox testing represents a cost-effective, rapid, sensitive and reproducible quality assurance and control to 1) detect polluters in the catchment, 2) safeguard optimal operation of the biological proecesses, 3) evaluate if 4th purifiaction steps are needed and which measures will be the most effective and 4) protect ecosystems. We present results from a 1 year online biomonitoring of the effluent (3 step purification process), followed by chemical diagnostics and ecotoxicological testing in alarm cases. For example, we could discern certain carbamates in the inflow to disrupt the biological purification and to affect gammarid survival in the Multispecies Freshwater Biomonitor. These carbamates show a certain rate of metabolisation during the purification process shown by chemical analytics and toxicity tests comparing inflow and effluent.

MO088
Ecotoxicological evaluation of inorganic fraction present in effluent delignification of linter produced by an explosive industry
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The effluents from explosive industries are potentially polluters. They can affect aquatic environments, due to the presence of toxic compounds, such as organochlorine compounds, lignin, 2,4,6-TNT and its metabolites , in addition to high concentration of inorganic ionic compounds. Often, with the treatment of these effluents, there is reduction of physico-chemical parameters such as BOD (biochemical oxygen demand), COD (chemical oxygen demand) and color, but the toxicity is not eliminated and in some cases may still suffer strong elevation. In this work, the effluent of the delignification of linter, generated from the production of nitrocellulose from an industry of explosives, was characterized chemically, for determination of pH, conductivity, turbidity, total phenols, color, BOD, COD, soluble and insoluble solids, total carbon, total organic carbon and total inorganic carbon. From this final analysis was generated a synthetic sewage containing NaCl, NaF, $CaSO_4.2\ H_2O$, $Ca(OH)_2$, KOH, $Mg(OH)_2$ and NaOH in the same industrial effluent concentrations. The synthetic sewage was then used in ecotoxicological tests with aquatic organisms. The chronic toxicity was determined using the green algae Pseudokirchneriella subcaptata and Ceriodaphnia dubia and the acute toxicity, using Daphnia similis. The results showed that the synthetic effluent presented high acute and chronic toxicity. The physical and chemical parameters analyzed showed high values of conductivity and pH, and may be considered as some of the factors responsible for toxicity. The inorganic fraction of the effluent of the delignification linter represents, therefore, a significant role in the toxicity of this, indicating that the same would need to go through a rigorous treatment to decrease this effect, thus avoiding large environmental impacts.
Acknowledgements: FAPESP and FEHIDRO

MO089
The effects of cadmium and zinc in a Flemish stream on the development and physiology of the zebrafish embryo.
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The production and commercialization of chemicals increases every year. Part of these substances end up in aquatic ecosystems, causing individual or mixed toxic effects. Unfortunately, risk assessment of environmental samples and effluents is almost solely based on chemical analyses, physicochemical characteristics and the existence of nutrients and micro-organisms. This results in the lack of information concerning the biological effects of complex environmental samples and effluents. Therefore, the goal of this study is to assess the biological effects of waste water and effluents by using the zebrafish embryo as a model. The advantage of this alternative method is that they are not considered as test animals until 120 hours post fertilization (hpf) and that the used volumes are small enabling the assessment of environmental samples in the laboratory. As the homeostasis range of zebrafish embryos for a lot of physicochemical characteristics of water is not yet known, this was tested prior to exposing embryos to environmental samples. Dose-response curves for both pH and salinity were made to detect the limits of the homeostasis of the embryos. Subsequently, zebrafish embryos were exposed to water samples of a Flemish industrial contaminated stream, 'Scheppelijke Nete', which contains high levels of cadmium and zinc. Sampling was performed upstream a pollution point (SN1), at a pollution source (DITCH) and downstream of the pollution source (SN2). The dissolved Cd and Zn concentrations were measured using ICP-OES. The highest concentrations of Cd and Zn were found in the DITCH and SN2 water respectively. The pH and salinity of the samples fell within the homeostasis range of zebrafish embryos The embryos were exposed to the samples and scored every 24h until 96 hpf for morphological and physiological parameters. The embryos exposed to SN2 and DITCH water had a delayed hatching and a lower hatching success compared to the control and the SN1 embryos. In order to determine if this effect was caused by Cd, Zn, a combined effect of both substances or by unknown substances, an experiment will be set up with pure Cd and Zn and with a combination of both at the same concentrations found in the samples, and compared with the hatching results of the three different water samples. In future research the zebrafish embryo will be further established as a model in risk assessment of environmental relevant samples such as river water samples and effluents.

MO090

The use of aquatic bioassays in the risk assessment to surface and groundwater from the application of organic wastes as soil improvers
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Abstract The Proposal for a European Soil Framework Directive recognized that "soil degradation or soil improvers have a major impact on other areas, (…) such as surface waters and groundwater, human health, climate change, protection of nature and biodiversity, and food safety". This study aims to assess the potential impact on surface and groundwater from the application of different organic wastes as soil improvers. For this purpose, sewage sludge, composted sewage sludge with agricultural wastes, agro-industrial sludge, mixed municipal solid waste compost (MMSWC), compost produced from agricultural wastes, pig slurry digestate (PSD), municipal slaughterhouse sludge, and paper mill wastes (sludge and ashes), were analyzed considering their main physicochemical characteristics, and their trace elements (TEs) total concentrations (Cd, Cr, Cu, Hg, Ni, Pb and Zn). In order to assess their potential impact on surface and groundwater, a leachate was obtained using DIN 38414-S4 method (1984). Several aquatic bioassays were used in order to assess the leachate ecotoxicity: luminescence inhibition of the marine bacteria *Vibrio fischeri*, *Daphnia magna* immobilization, *Thamnocephalus platyurus* survival, and seed germination (*Lolium perenne* and *Lactuca sativa*). Considering the total TEs concentrations in the organic wastes, and their organic contaminants concentrations, the sludge samples characterized in the study can be used for agricultural purposes, in accordance with the European Community Directive 86/278/EEC and with the Portuguese legislation. As for the compost samples, they can also be land applied considering the DG Env.A.2. Working document on "Biological Treatment of Biowaste – 2nd draft": mixed municipal solid waste compost is a Stabilized Biowaste, composted sewage sludge with agricultural wastes is a Class I compost. However, compost produced from agricultural wastes and pig slurry digestate cannot be land applied, due to their Ni and Zn content, respectively. Despite the fact that some organic wastes are allowed to be land applied according to the existing regulations, their leachates can be very toxic towards some organisms. That toxicity was difficult to be predicted from the total TEs concentrations, which are one of the main criterions to be accomplished when evaluating organic wastes risk – PSD and MMSWC were the organic wastes with overall higher total TE concentrations and were not identified as having higher toxicity.

MO091
Effect-directed investigations of selected effluents and receiving waterbodies in the Meuse and Scheldt basins: overall toxicity and endocrine disruption assessment.
Y. Marneffe, Inst Scientific de Service Public / Ecotoxicology Department; C. Chalon, ISSeP
Bioassays are a holistic and meaningful way of assessing effects of environmental samples and wastes on ecosystems. A study was carried out with complementary tools in different rivers of the Meuse and Scheldt basins (water and/or sediments), upstream, downstream and in the effluent of selected industries and urban wastewater treatment plants. An effect directed active monitoring using bioassays was carried out, combining ecotoxicological and physico-chemical measurements. A yeast estrogen screen (YES) assay was conducted as effective first-tier screening tool to assess the estrogenic potential of surface water, effluent or sediment samples. Moreover, we used a battery of short term and chronic bioassays with the bacteria *Vibrio fischeri*, the alga *Pseudokirchneriella subcapitata*, the rotifer *Brachionus calyciflorus* and the microcrustacea *Daphnia magna* to assess their overall toxicity. Polar organic chemical integrative samplers (POCIS) were also used at some locations to sample hydrophilic compounds such as pharmaceuticals, personal care products, hormones, and pesticides having low log *n*-octanol-water partition coefficents (Kows < 3). POCIS extracts were screened for the presence of estrogenic chemicals using the yeast estrogen screen (YES) assay and analyzed by gas chromatography/mass spectrometry (GC/MS) and we used AMDIS (Automated Mass Spectral Deconvolution and Identification software) and the NIST 2.0 mass spectral library for the identification of the compounds. Results from the YES indicated important estrogenicity in different small rivers such as River Haine and River Hain (>15 ng eq E2/l), whereas samples collected in more important rivers like the River Meuse showed lower estrogenic activities (

MO092
Making wood energy sustainable – Ecotoxicological study of wood ash from untreated fuel
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When it comes to sustainability perception of energy from biofuels, carbon budgets are the predominant concern, but accruing ash waste is still seldom considered. Whereas in some countries wood ash is used for fertilization and liming, in most counties the ash is still regarded as waste and its after-use hampered by the lack of ecotoxicological data. The current practice of ash disposal is a widely acknowledged weakness in the use of biofuels. A battery of standard OECD aquatic toxicity tests (representing all trophic levels) was applied to assess the effects of bottom and fly ash accruing from a power plant fired with untreated wood fuel. The results show that (1) bottom ash samples exhibited lower toxicity than fly ash, (2) alkalinity was a major contributing factor in overall ash toxicity, (3) although EC_{50} values were established within one order of magnitude (e.g. native bottom ash EC_{50} in algal growth inhibition, Microtox, duckweed growth inhibition, daphnia immobilization and fish survival tests were 3.7 $g{\cdot}L^{-1}$, 4.8 $g{\cdot}L^{-1}$, 36.9 $g{\cdot}L^{-1}$, 55.5 $g{\cdot}L^{-1}$ and 97.8 $g{\cdot}L^{-1}$ respectively) they appeared to increase with the complexity of the biological models (4) while pulverization of the samples had little effect on their toxicity, re-ignition of the ash resulting in complete mineralization led to an increase of toxicity probably due to increased mobility of elementary components and their effect on the sample pH. In general fly ash is considered to pose a considerably greater hazard than bottom ash. Although our data confirm higher toxicity of fly ash, the observed differences in EC_{50} values for the two ash types from untreated wood fuel are not substantial (e.g. EC_{50} for native bottom and fly ash in algal growth inhibition and Microtox tests were 3.7 and 1.9 $g{\cdot}L^{-1}$, 4.8 and 3.6 $g{\cdot}L^{-1}$ respectively). The findings also illustrate that an increased degree of combustion reduces the level of organic contaminants and results in increased mobility of mineral components and higher pH. The presented data will facilitate the risk assessment for after-use of ash from untreated wood fuels in Ireland, might support the reconsideration of ash fate and thereby increase the actual sustainability of wood biofuels.

MO093
Toxicity assessment in photocatalytic degradation of BPA using nano-sized and mesoporous TiO2
J. Jung, Korea University / Professor; A. Jo, Korea University; Y. Park, University of Seoul
Advanced oxidation processes (AOPs) including Fenton reaction, UV photocatalysis and ozonation have been widely studied in treatment of refractory organic pollutants. However, most studies have focused on removal efficiency of the organic pollutants without toxicity assessment of their degradation products. In the previous study, we found that acute toxicity of phenol and monochlorophenols to *Daphnia magna* was dramatically increased by gamma-ray treatment doses as low as 1 kGy. Thus, the aims of this study were: (1) to investigate photocatalytic degradation of bisphenol A (BPA) using the commercial Degussa P25 (NP-TiO_2) and hierachically mesoporous TiO_2(HM-TiO_2); and (2) to evaluate the changes in toxicity toward *Vibrio fischeri* (luminescent bacteria) during the treatment. Photocatalytic treatment in the presence of NP-TiO_2 was efficient to remove BPA ($1{\times}10^{-5}$ M), thus around 95% of BPA was degraded within 64 min. Meanwhile, photolysis, hydrolysis and adsorption processes were not significant compared to the photocatalytic reaction in this treatment. With the HM-TiO_2, the efficienty of photocatalytic treatment was decreased to some degree, thus around 94% of BPA was removed in 180 min. Acute toxicity of BPA to *V. fischeri* was dramatically increased by photocatlytic treatment with NP-TiO_2, where toxicity was gradually decreased in the presence of the HM-TiO_2. The different toxidity results are likely related to different formation of intermediates in the photocatalytic treatment, and this should be further identified. Photocatalytic treatment seemed to be effective in degrading BPA, but the removal rate and toxicity change largely depended on the property of catalysts used. For instance, degradation of BPA with the commercial Degussa P25 (NP-TiO_2) gave an extraordinary toxicity increase. Therefore, the reaction mechanism and by-products should be fully characterized to idenfify the cause of toxicity in the photocatalytic treatment.

MO094
Molecular biomarkers in oysters Crassostrea gigas exposed in situ to sanitary sewage discharges - Five years of studies
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Among the main causes of the coastal contamination around the world are the discharges of untreated sanitary sewage, which has chronically affected the health of aquatic organisms. In view of the ecologic and economic importance of oysters to coastal areas, understanding their biology and susceptibility to these pollutants have become an important issue in ecotoxicology. In particular, transcriptomic studies have received special attention due to the enhanced possibility of elucidate different mechanisms of adaptation to contaminant exposure. The aim of this study was to identify genes from *Crassostrea gigas* as candidate to be used as biomarkers of exposure to the complex mixtures of sanitary sewage in the real world situation.

Since 2008, annually oysters have being caged at sites contaminated by sanitary sewage discharges and at two reference sites (areas used for oyster culture) in Santa Catarina Island, south of Brazil. Levels of linear alkyl benzenes (LABs), polycyclic aromatic hydrocarbons (PAHs) and fecal steroids were analyzed in tissues of oysters before and after the exposure in all sites. The mRNA from gill and digestive gland were extracted, purified and the cDNA was synthesized according conventional protocols. qPCR were carried out to quantified the levels of transcription of previously identified genes as *fatty acid binding protein* (*FABP*), *glutathione S transferase omega class* (*GSTO*) and different *cytochrome P450 isoforms* (*CYP356A1*, *CYP2AU2*, *CYP30C1*). The experiments carried out along these five years showed a consistent induction of transcription of FABP, GSTO and these CYPs in gills of oysters exposed to sanitary sewage discharges, comparing to the oysters kept at the reference sites. These data are corroborated by the significant levels of bioaccumulation of LABs, PAHs and fecal steroids in the tissues of oysters. Based on these data, we suggest the use of these early warning molecular tools as complementary techniques to monitor the impact of sanitary sewage discharges in coastal areas. Supported by CNPq, INCT-TA.

MO095

THE TOXICITY OF LEACHATES FROM INDUSTRIAL WASTE CONTAINING ANTIMONY

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Antimony (Sb) is a metalloid with broad industrial applications. Despite the wide use of antimony and associated compounds there exists a lack of reliable information with regard to the environmental toxicity of antimony and associated compounds and their effects in aquatic ecosystems. The study investigated the toxicity of 'artificial' leachates from an EPA supplied industrial dry waste sample, known to contain high levels of Sb.. Inductively Coupled Plasma Mass Spectrometry (ICPMS) was used to quantify the levels of Sb present in the sample. Two bioassays were then conducted to assess the toxicity of this leachate, comprising (1) a MicrotoxÒ test using the luminescent bacteria *Vibrio fischeri,* and (2) a test utilising the sensitive freshwater micro invertebrate *Hydra* spp. ICPMS analysis of the produced leachates determined an antimony concentration of 10.51 and 106.09 mg/L in acidic and alkaline mediums, respectively. Bacterial testing using the MicrotoxÒ acute toxicity test protocol recorded a fifteen-minute EC_{50} value of 36.4 mg/L for Sb solution. The leaching fluid of pH 9.2 was the most toxic of all leachate solutions tested to *Vibrio fischeri* and its toxicity increased with time. In contrast, the toxicity of the leachates at pH 2.9 showed little change with time over 15 minutes. 96 hour acute toxicity testing was also performed using the freshwater cnidarian *Hydra*. 96 hourEC_{50} and LC_{50} values for antimony solution were 0.698 mg/L and 1.66 mg/L, respectively. The acidic leachate was less toxic to *Hydra* sp. with a 96 hourEC_{50} and LC_{50} values of 3.25 % and 4.42 % (v/v of the original solution), respectively, while the alkaline leachate had EC_{50} and LC_{50} values of 1.11 % and 1.77 % (v/v of the original solution), respectively. . Comparison of determined toxicity endpoints with an aquatic hazard classification scheme suggests that antimony had a similar toxicity to arsenic and that at the very least, antimony has the potential to cause harm to aquatic biota.

MO096

DETERMINATION OF TOXICITY OF WASTEWATER IN SLAUGHTERHOUSE ORIGINATED IN THE BENEFICIATION PROCESS OF BEEF

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The Brazil is a great producer and exporter of meat. During the processing of meat are used large amounts of water, leading to generation of high volumes of wastewater from slaughterhouse. As altas concentrações de matéria orgânica, formas nitrogenadas e fósforo, aliada a diversidade de íons, tornam esse efluente propício a ser tratado por processos biológicos. The high concentrations of organic matter, nitrogenous and phosphorus forms, added to a high diversity of ions, make this effluent able to be treated by biological processes. The presence of organic compounds such as carboxylic acids, aldehydes and alcohols, may cause toxic effects and harmful to the aquatic environment. The present study aimed to evaluate the toxic potential of wastewater from slaughterhouse from the green line, generated iby processing of beef of a fridge, located in the region of Vale do Paraíba, SP, Brazil. The organisms used for the acute toxicity tests were the microcrustáceo *Daphnia similis* ($EC_{50\ 48\ h}$), the bacteria *Pseudomonas putida* ($EC_{50\ 6:0}$) and *Escherichia coli* ($EC_{50\ 6:0}$). For chronic toxicity test were used the algae *Pseudokirchneriella subcaptata* ($LC_{50\ 96\ h}$) and microcrustaceans *Ceriodphnia dubia* and *Ceriodphnia silvestri* ($LC_{50\ 192\ h}$), OEC, NOEC and CV. The results showed the effluent toxicity to tested organisms. The EC50 values were 1.56% (1.28 -1.89), 4.47% (93.75 -5.33) and 39.77% (33.47 -47.19) for *D. similis*, *P. putida* and *E. coli*, respectively. The tests using the *C. dubia* and *C. silvestri* showed chronic toxicity to the effluent, with values of less than 0.3% NOEC and LC50 of 1.16% and 0.56%, respectively. For *P. subcaptata* the NOEC values were of 4.17 and LC50 of 7.3%.The evaluation of physico-chemical parameters revealed that the effluent had high concentrations of total solids, total volatile acids, organic matter, nutrients and alkalinity, that may be the agents of toxicity. According to the results obtained, the effluent tested is toxic and its launch, without treatment, in bodies receivers could compromise the aquatic life. ***Acknowledgement:***Fapesp, CNPq e Capes

MO097

Implementation of the test-battery approach into routine effluent control in Lithuania

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Recently, HELCOM, expressing a concern on the status of the Baltic Sea, has initiated several international programmes such as Cohiba, BaltHaz, etc., particularly targeted to promote the usage of Whole Effluent Assessment (WEA) methods in routine regulation. Contrary to the control of individual substances, which is based on knowledge of a single concentration, municipal and industrial effluents can be regulated directly on basis of harmful effects that occur in testing organisms. Various countries use laboratory toxicity tests to monitor effluent discharges into surface waters in various degrees. For example, acute and chronic toxicity tests are used in the USAand Sweden, whereas acute tests are used in Canadaand several European countries. Up to date, the majority of countries provide chemical-based effluent controls, however, the progressive increase of chemicals under control and difficulties in measuring them make individual-substance approach problematic. In some countries, toxicity data are included for taxation purposes, in parallel to chemical-based taxation. For example, toxicity data obtained from *D. magna* acute and fish embryos tests used inFrance andGermany, respectively. In 2011, the Ministry of Environment of Lithuania put forward a national programme with the aim to elaborate a system of effluent control by implementing toxicity tests. The outcome of this programme includes appearance of necessary environmental juristic documents and/or their updates, selection of test-batteries, selection of the cumulative index for toxicity evaluation and performance of initial stage of effluent inventory monitoring. The evaluation of potential harm for aquatic environment is limited to the application of end-of-pipe principle, the assessment of effluent by acute and/or chronic toxicity test-batteries as well as effluent-and stream-flow rates. The polluters will be grouped according to the criteria of the type of economical activity and discharge rate. During one-year inventory period, respective test-battery(ies) will be applied and then the most sensitive test(s) prescribed for routine effluent control. In this study, a system for municipal and industrial effluents control as well as simultaneous steps of its implementation into routine will be overviewed.

MO098

Impact of wastewater treatment plant effluent on Gammarus fossarum´s feeding rate and vitellogenin levels

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Wastewater treatment plant (WWTP) effluents release complex mixtures of organic and inorganic micropullutants, including endocrine disrupting compounds, into receiving water bodies. These substances may cause adverse effects in aquatic communities. To uncover potential implications on the endocrine system of crustacean species as well as on their part in ecosystem functions, i.e. leaf litter decomposition, the key shredder *Gammarus fossarum* was exposed four times during April – May 2012 for one week periods up- or downstream of a WWTP located close to Zurich, Switzerland. Additionally, the induction of vitellogenin (vg) was assessed in animals exposed to Cyproterone as reference chemical and wastewater *in situ* and in the lab. Furthermore, estrogenic activity of the river water was measured. For this purpose, extracts from passive samplers, which were installed in the receiving stream during in situ experiments, as well as water samples taken once per week were analyzed using a yeast estrogen screen (YES) assay. Additional water was analyzed for transformations products. The gammarid´s feeding rate was significantly reduced 100, 200 and 400 m downstream of the WWTP relative to the 100 m upstream site. While YES results showed elevated estrogenicity at downstream sites, vg concentrations in amphipods were not significantly changed. This lack of responsiveness was corroborated in lab experiments. In conclusion, the results of this study indicate that wastewater released into natural aquatic ecosystem may considerably affect the ecosystem function of leaf litter breakdown, while effects on the endocrine system of crustaceans were not detectable.

MO099

Toxicity of solid wastes formed and released after wastewater treatment with nanomaterials

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Nanomaterials (NMs) are being widely recommended for water and wastewater treatments due to their unique physical and chemical properties. Several studies reporting the different advantages of nanotechnology in the remediation of wastewaters are numerous in the literature, but limited research effort has been directed toward understanding the fate and potential impacts of the solid residuals, that could persist in the treated effluent, being accidentally released to the receptor freshwater ecosystems, after the application of such technologies. The treatment of wastewaters containing organic and inorganic contaminants with NMs will result in solid wastes containing NMs, used for the treatment, and several chemicals bound to them. It is then imperative to assess the environmental safety of these wastes. The present work aimed at investigating the ecotoxicity of solid wastes resulting from the treatment of three effluents (olive oil mill, kraft pulp mill, and mining drainage) with two (TiO_2 and Fe_2O_3) NMs. Since these residues will eventually accumulate in the sediment of the aquatic compartment, the invertebrate *Chironomus riparius* was selected as test organism and exposed to the residues. A 10-day static laboratory bioassay was performed by exposing first instar larvae to artificial sediment spiked with solid residues of nano-TiO_2 (22.7-38.0 mg TiO_2 Kg^{-1} d.w. of soil) and nano-Fe_2O_3 (21.3-35.3 mg Fe_2O_3 Kg^{-1} d.w. of soil) resulting from the treatment of each effluent. These solid residues were chosen from previous work, from the treatments with best performance in ameliorating the chemical characteristics and the toxicity of each effluent. The effect on percentage of survival and growth (measured body length and extrapolated from the width of the head capsule) was assessed. Results showed that the residues from the treatments TiO_2/H_2O_2 and Fe_2O_3/H_2O_2 from olive oil mill effluent and Fe_2O_3/H_2O_2 from kraft paper mill effluent did exhibited lethal toxicity to *C. riparius* larvae, as the percentage of survival reached 60%. Only the exposure with residues from the treatment Fe_2O_3/H_2O_2 applied to the kraft paper mill effluent significantly affected the growth rate extrapolated from the head capsule width. In terms of growth rate computed through the measures of body length, it decreased significantly after exposure to the residues from the treatments TiO_2 (1.0 g L^{-1}) and Fe_2O_3/H_2O_2 of kraft paper mill effluent and Fe_2O_3/H_2O_2 of olive oil mill effluent.

MO100

Which chemicals drive biological effects in wastewater and recycled water?

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Compliance monitoring of drinking water and recycled water in Australia is predominantly based on chemical assessments using instrumental analysis. Bioanalytical tools have the potential to assess the mixture effects according to the mode of toxic action and can complement chemical analytical monitoring. In this study, grab samples were collected from an Australian Wastewater Treatment Plant (WWTP) with secondary treatment processes, including activated sludge treatment, followed by treatment in an Advanced Water Recycling Plant (AWRP). Advanced treatment included ultrafiltration, chloramination, reverse osmosis (RO) and UV disinfection. Analysis of 278 compounds was undertaken at different points along the treatment train. Treatment efficiently removed most organic compounds mainly by reverse osmosis. Detected analytes after RO were below the Australian Guidelines for Water Recycling (AGWR) and as such would not be expected to pose any health risk and complete removal was observed in the post-UV water. In parallel, a battery of cell-based bioassays covering a wide range of modes of action were used to evaluate the samples. In laboratory studies, identified chemicals were grouped into major categories (including pesticides, herbicides, pharmaceuticals, endocrine disrupting compounds and X-ray contrast media) and were mixed in the concentration ratios they were detected in water samples prior to RO. The mixtures were dosed into the bioassays. The effects caused by these designed mixtures were compared to the effects of the corresponding entire samples. Generally antibiotics dominated the mixture effects in WWTP effluent, while post RO pharmaceuticals and pesticides became more important. The contribution of the type of chemical to overall effect was also dependent on the toxicity endpoint. For receptor-mediated biological endpoints such as photosynthesis inhibition, where a small number of well-defined chemicals are known to be active, the majority of effects could be explained by the presence of identified compounds. For non-specific bioassays such as cytotoxicity or oxidative stress response, where all or many compounds contribute to the mixture effects, the detected chemicals could explain less than 1% of the measured effect, meaning that non-target chemicals and transformation products contribute to the mixture effects.

MO101

Establishment of an elution method of three beta-blockers for aquatic ecotoxicity testing with pills

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Pharmaceuticals and their metabolites have been emitted in large amounts to the aquatic environment for many years, mostly as complex mixtures in the effluents of sewage treatment plants and sludge. One of the most important groups of prescription drugs are beta-blockers, which play a significant role for the therapy of cardiovascular diseases. Three of the major active ingredients used in Germany are: atenolol (55.5 million defined daily doses, DDD), bisoprolol (659 million DDD) and metoprolol (892.2 million DDD). As a consequence of high volume of usage, beta-blockers are likely to have a more or less constant presence in the aquatic environment, detected in the influent and effluents of sewage treatment plants as well as in rivers. For the assessment of the ecotoxicological relevance of atenolol, bisoprolol (as bisoprolol hemifumarate) and metoprolol (as metoprolol tartrate) in the aquatic environment, the acute *Daphnia magna* immobilisation assay according to ISO 6341 was used. Due to testing of unknown pharmaceuticals as pure substances in analytical grade is often very expensive for EC_{50}-range-finding, an elution method with pills of these beta-blockers was established. For the assessment of a suitable elution method, primarily by acute data, definition of stock solution for testing was carried out according to ECOSAR using SAR for aliphatic amines of beta-blockers as baseline toxicants. For comparison of two different elution methods, stock solutions with a total of 500 mg pills per litre water were prepared. Immobility of *D. magna* was observed after 24 and 48 h with the latter being the endpoint for effect calculation. For chemical and ecotoxicological characterisation of filtered water extracts, EC_{50}-range-finding was performed in combination with chemical analysis by detection of compound content using LC/MS. For evaluation of a suitable elution method with pills, the recovery rate of atenolol (83.6 %), bisoprolol (93.8 %) and metoprolol (76.2 %) was calculated by the measured compound content in the water extracts. Risk classification was carried out by means of observed EC_{50}-values according to Technical Guidance Document. Metoprolol would be classified as harmful to aquatic organisms (10-100 mg/L), while atenolol and bisoprolol would be classified as non-toxic (>100 mg/L). These investigations reveal a suitable elution method with pills for the environmental relevance of unknown pharmaceuticals and their metabolites in sewage treatment plants.

MO102

Is the zebrafish embryo test a suitable tool to evaluate the efficiency of wastewater treatment plants?

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\n The entry of various bacteria and micropollutants in surface waters occurs to a great extent through wastewater treatment plants (WWTPs) and storm-water overflow systems. Both incomplete purification of wastewater in WWTPs and low storage capacity of stormwater overflow basins (SOBs) during heavy rain events contribute to this. As a consequence, micropollutants emerge in surface waters and partly accumulate in sediments. This can result in negative consequences on the development of fish and other aquatic organisms in the affected surface waters. By upgrading the WWTPs with additional purification steps such as ozonation, sand filtration and activated carbon filters, it is possible to improve the elimination rates of micropollutants and pathogens. The joint project "SchussenAktiv*plus*" investigates the efficiency of three WWTPs of different size and two types of stormwater overflow basins (SOBs) before and after the startup of additional purification steps. For this purpose, various chemical and microbiological analyses and biological *in vitro* and *in vivo* tests are conducted by several co-operation partners. The present part of the project focuses on the investigation of the cleaning efficiency of WWTP and SOB upgrading with regard to potential developmental toxicity of treated waste water. For this, wastewater is investigated before and after passing the additional purification steps by using a zebrafish (*Danio rerio*) embryo test. The three examined test systems in this project are the WWTPs Langwiese, Eriskirch and Merklingen and the SOBs Mariatal and Tettnang (Germany). The WTTPS Langwiese and Eriskirch and the SOB Mariatal are located at the Schussen River, a tributary of Lake Constance. No significant differences were found between the conventional cleaning and the additional purification steps with respect to embryotoxicity, but some tendencies towards less pronounced adverse effects after WWTP upgrading became obvious. Compared to embryotests with trout which can be conducted in the field, the zebrafish embryotest only provides information on embryotoxic potentials in environmental samples. It is easier to conduct but generally less sensitive.

MO103
Evaluation of DEET cytotoxicity on Perna perna mussels
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Recent studies have identified the presence of several emerging pollutants in aquatic environments. The occurrence in different environmental matrices has been continuously reported, highlighting the need for toxicity studies. Developed in the 1940s and present in many commercially available formulations, the DEET (*N,N-diethyl-meta-toluamide*) is the active agent used in most insect repellents. Several studies have been identified the DEET presence in surface water and in wastewater treatment plant (WWTP) effluent, so this compound is considered an emerging pollutant and studies indicate that DEET is only slightly toxic to aquatic organisms. Even nowadays the environmental destiny of DEET is not completely understood. Although DEET is considered a compound resistant to degradation and commonly found in surface water, there are not complete assessments for ecological risk of DEET, including studies evaluating chronic toxicity to aquatic organisms. In this study was evaluated the DEET toxicity and the effects caused in lysosomes of *Perna perna* mussels hemocytes. For this purpose, firstly was performed the acute toxicity assay to identify the DEET concentration that causes 50% mortality of exposed organisms to DEET concentrations in the range of 75 to 400 mg L^{-1} and the obtained lethal dose (LC50) was 114.27 mg L^{-1}. To assess the stability of the lysosomal membrane in organisms exposed to this compound was carried out the cytotoxicity assay utilizing DEET concentrations in a range of 0.001 to 1.0 mg L^{-1}. In fact, is important reinforce that the data of DEET cytotoxicity showed disturbances in *Perna perna* mussels in all tested concentrations.

MO104
Phytotoxicity assessment of discharge waters: Focus on germination and root elongation tests
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Surface Treatment Industry is well known to be one of the largest chemicals and water consumer. It thus generates a large amount of complex and toxic waste water. Seed germination and root elongation tests, using as indicator higher plants like cucumber, radish, wheat and lettuce, are commonly used to evaluate their ecotoxicity. Indeed these bioassays present many advantages as they are simple, very reproductible, rapid and only require a small amount of sample. However these tests are carried out under national and international recommendations (US FDA, US EPA, OECD), some parameters remains variable. Our research group historically uses the lettuce *Lactuca sativa* (L.) as bio-indicator in ecotoxicological bio-monitoring. The aim of this study is to assess which factor(s) can be seen as critical in the water discharge toxicity evaluation. We particularly focus on control water quality, seed abundance or lettuce variety. We tested four waters as control (mineral, osmozed, ultra pure and distilled water) and three abundances (15, 20 and 30 seeds per Petri dish). After 7 days in controlled conditions (dark; 24°C±1°C), results show no significative differences on both germination rate and root elongation endpoints. Nevertheless, we find out that when watered with industrial waste water, the four lettuce varieties (Batavia dorée de printemps, Kinemontepas, Appia and Grosse Blonde Paresseuse), randomly chosen among more than 1500 commercial cultivars, show significatively different responses. From the comparison, it is clearly evident that a differential sensitivity scale exist among nor species but varieties.

MO105
Toxic potential of different types of sewage sludge as fertilizer in agriculture: ecotoxicological effects on aquatic and soil indicator species
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Phosphorus is a non-renewable, essential but limited resource for plant growth. Waste water and sewage sludge usually contain substantial concentrations of phosphorus. In order to use sewage sludge as a sustainable phosphorus resource for agriculture, it has to be proven that no ecotoxicological effects on target species in soil and receiving water bodies are generated. Therefore, three test species were chosen to cover the environmental compartments water, sediment and soil. To evaluate acute effects of sewage sludge on a higher aquatic plant, the duckweed *Lemna minor* was monitored via its growth inhibition, discoloration and colony break-up. Acute toxicity of sewage sludge in water and sediment was investigated with regard to mortality and behavior (movement activity and feeding behavior) of *Gammarus fossarum*, a key organism in stream ecosystems. The toxic effects of sewage sludge in soil on a decomposer - the earthworm (*Eisenia fetida)* - were monitored by its avoidance behavior. Chemical assessment included nutrients, organic micropollutants and heavy metals. \n For example, the assessment of a non-dewatered sludge resulted in an inhibition of growth of *Lemna minor* starting from 2,5 volume-percentage after 7 days (EC_{50}: 5,1% S). Concentrations below 1% supported plant growth (hormesis effect). *G. fossarum* displayed significantly decreased movement activity at 2 and 5% sludge concentration during an exposure time of 2 days, leading to decreased survival after 4 days of exposure in 2% sample concentration (LC_{50}: 1,95% S). After 2 days *E. fetida* exhibited an increased avoidance behavior of contaminated soil from 1% sewage sludge (EC_{50}: 1,7% S). 100% avoidance of contaminated soil was obtained at 5%. The dewatered sludges had a higher toxic effect on *E. fetida* and *G. fossarum* but a lower toxic effect on growth inhibition of *L. minor*. *G. fossarum* was the most sensitive species in the applied test setup.\n In conclusion, for all three test species all three sewage sludges tested have to be classified as toxic at high concentration levels under laboratory conditions. In order to get reliable information about practical doses for agricultural soil, field studies under real environmental conditions will be performed to verify the current results.\n The research project P-REX is supported by the European Commission under the Seventh Framework Programme (Priority: "From Prototype To Market"/ Contract No. 308645).

MO106
LogNormality of trace contaminant concentrations in sewage effluents
M. Gardner,
Summary: This poster summarises a detailed examination of the nature of the data frequency distributions of trace contaminant in wastewater treatment works' effluents. Cumulative distribution plots are illustrated for both between-works average values and within over 150 works. **Abstract:** It is important to understand the statistical distribution of monitoring data for them to be of value in determining the parameters of environmental models. No such distributional information has been available for many trace contaminants in sewage effluents. This work applies the data of a major UK sewage works' effluent monitoring programme to determine the validity of the common assumption that data are logNormally distributed. Effluent quality was monitored at 162 wastewater treatment works over one year, generating over 3,000 results for each of over forty substances, including metals, trace organic substances, pharmaceuticals etc. It is demonstrated that the logNormal assumption is clearly justified for the great majority of substances in the spatial case - for annual average effluent concentrations across different treatment works. In the site specific, temporal case – for individual determinations of concentration at a single site over an annual period – logNormality is generally supported, but not demonstrated so unequivocally for all site/substance combinations. The principal source of uncertainty for within works data was lack of sufficient numbers of observations reported to adequately low reporting limits.

MO107
Detection and fate of synthetic musks in wastewater treatment plants – a review
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Synthetic musks are used as fragrance additives and fixative compounds in personal care (e.g. perfumes, lotions, shampoos, deodorants) and household products (e.g. detergents, fabric softeners, air fresheners). Due to their high incidence and widespread use, synthetic musks are continuously introduced into the environment, mainly through urban sewer systems. Since these compounds are pseudo-persistent, bioaccumulative, have a lipophilic nature and are only partially biodegradable, they are usually not completely removed when they reach wastewater treatment plants (WWTPs). Therefore, their effluents are the main source of contamination. In fact, the use of biosolids as fertilizers in agricultural fields is a direct input of musks into the soil, whereas the wastewater effluents discharges are the major route for surface water contamination. To the author's best knowledge, this is the first compilation of studies about concentration levels and fate of synthetic musks in WWTPs and discusses the efficiency of the traditional removal methodologies applied in these plants. In this study, it was seen that polycyclic musks are the most detected, namely galaxolide (HHCB), tonalide (AHTN) and DPMI (cashmeran). They are found in effluents at concentrations from 5 to 10525 ng/L. Nitromusks are detected in a small number of effluents, but when detected, their concentrations ranged from 0.3 to 542 ng/L in wastewater. The most detected are musk xylene (MX) and musk ketone (MK). As expected, due to their lipophilic behaviour, musk compounds tend to accumulate mainly in sludge (0.05 - 117000 ng/g). Although synthetic musks were studied throughout the entire WWTP system, only a few studies dealt with measurements of the surrounding air. These revealed that musk compounds tend to accumulate in the gas-phase (0.23 – 344306 ng/m^3), being HHCB and AHTN the prevailing ones. Looking at the different stages of a traditional WWTP, secondary treatment is the process that most contributes for the removal of these kind of compounds, mainly due to sorption onto sludge. Acknowledgements The authors wish to thank Fundação para a Ciência e a Tecnologia (FCT - Portugal) for the project PTDC/AGR-CFL/102597/2008 and grant SFRH/BPD/76974/2011. This work has been partially funded by the European Union Seventh Framework Programme-Marie Curie COFUND (FP7/2007-2013) under UMU Incoming Mobility Programme ACTion (U-IMPACT) Grant Agreement 267143.

MO108

Record sediment concentration of retene, a highly potent fish teratogen, is buried into a lake site near closed pulp factory in Fennoscandia
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Lake Lievestuoreenjärvi (LLTJ) with the pulp factory on its shore, closed in 1985, is a national landmark of environmental history in Finland. During years of its operation since 1927, primarily by sulphite process , production of chlorine bleached softwood pulp resulted effluent discharges to LLTJ. In 2009, we sampled a 30 cm cores from the deposited sediment in the nearest deep (19 m), and sliced it into 2.5 cm subsamples for GC-MS analyses of wood- and municipality-derived chemomarkers. The highest concentration of retene (5765 μg/g dry w.) was found in the layer 10-12.5 cm, similar to the maximum of total resin acids, RAs (3532 μg/g; dominated by dehydroabietic acid, the primary precursor of retene). The uppermost core section, above 7.5 cm, contained dramatically lesser concentrations of any wood-derived extractive. While microbial aromatization of RAs into retene (7-isopropyl-1-methyl phenantrene) requires anaerobic conditions, we suggest that, besides evoked serious hypoxia due to pulpmill effluents, the domestic sewage discharged from the nearby village Lievestuore (ca. 3000 inhabitants) has maintained over decades the formation of retene inside burying sediment layers. This deduction is supported by synchronic presence of cholesterol and coprostanol , two human origin sterols, in the same layers with industrial wood extractives.

MO109
Automated detection of suspected and non-targeted metabolites in sewage water after biological and chemical treatment
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Water purification and treatment of sewage water is a well-known topic to the scientific community for a long time. Nevertheless, the question of the fate of contaminants in the different stages of treatment remains a complex matter and an analytical challenge. Triple stage quadrupole mass spectrometry equipment enables analytical chemists to conduct searches for known and suspected metabolites and transformation products, but separation from matrix signals stays critical and result confirmation difficult. The ongoing development of high resolution accurate mass (HRAM) mass spectrometric instrumentation (Orbitrap, TOF) opens up the door to more sophisticated ways of detection and confirmation of contaminants and their metabolites and transformation products, respectively. With these instruments, the detection mostly is done in full scan experiments, accompanied by different types of fragment scans for additional processing and confirmation. The power of resolution serves for unambiguous separation from the surrounding matrix signals, while mass accuracy is the key for reliable identification, including the isotope pattern for confirmation. Since all steps of detection, identification and confirmation take place post acquisition, the bottleneck is found to be the ability to process large amounts of data in appropriate time. For this study we took different water samples from a waste water treatment plant in western Germany with biological and chemical (ozonation) treatment facilities. We show how data, acquired with bench top Orbitrap HRAM LC-MS instrumentation can be analyzed in a fully automated matter with Thermo Scientific Compound Discoverer™ software. We show how the targeted search for known and suspected metabolites and transformation products by application of known and postulated biological and chemical transformation steps can be carried out easily in short time. Additionally, we show the detection of putative transformation products unknown so far by application of a generic component detection algorithm and application of mass defect filtering for specific parent components.

MO110
UKWIR Chemicals Investigation Programme – Phase 2 - from generic assessment to specific characterisation of effluent quality
M. Gardner; A. Thornton, Atkins Limited; L. Wilson,
Summary: This poster summarises the developing approach adopted in the UK to challenges posed by management of chemical in wastewaters. Where previous investigation focused on characterisation of effluent quality in terms of the presence and variability of substances in effluents, work currently in progress has moved in the direction of action to manage contamination. **Abstract:** Phase 1 of the UKWIR Chemicals Investigation Programme has provided a generic overview of trace contaminant concentrations in UK wastewater treatment works effluents, the effectiveness of current treatment processes in reducing contaminant concentrations and a novel insight into the sources of substances in sewer catchments. The second phase of this programme (CIP2) has been developed as an extension and expansion of an already substantial (€30M) CIP1 programme. The risk-based approach that has been widely used to scope the scale of measures required to meet Environmental Quality Standards (EQSs) for trace contaminants must now be supplanted with a clear view of actual environmental status. In order to meet this challenge, it is necessary to gather a suitably substantial and reliable body of evidence relating to compliance with quality standards. The intention is that resources expended in these investigations will ensure that the potentially far greater investment in possible remedial action will be directed where it can be shown that there is a demonstrable need and where responsibility can be apportioned such that the "polluter pays". Equally importantly, the data from CIP2 should make it possible to differentiate between sites where the case for action is clearly demonstrated and those where it is weak or non-existent. The way in which monitoring will be supported by definition of clear strategies for stakeholder engagement in substance source control and scoping of further improvement or upgrading of wastewater treatment processes, will also be described.

MO111
Persistence of wastewater-related xenobiotics during transport along an urban river segment
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Xenobiotics are increasingly produced by industrial processes and introduced into the environment. Many of them are not completely eliminated by conventional waste water treatment plants (WWTP) and enter the receiving waters by WWTP outfalls or combined sewer overflows. In many cases, little is known about their toxicity, persistence and transport behavior in aquatic systems. In this study, the behavior of selected organic pollutants along a 4 km long urban river segment was studied by an experimental approach. The Steinlach River in southwest Germany with a total catchment area of 140 km^2 receives treated wastewater from a WWTP a couple of kilometers upstream of its confluence with the Neckar River. In its further course, the river channel is largely straightened and does not receive any larger tributaries. For this segment, a detailed mass balance was determined over a complete 24 h cycle. To this end, 2 h composite samples (sampling interval: 15 min) were taken using automated samplers at the upstream and downstream ends of the segment, respectively, and analyzed in the lab. A model-based analysis of the data demonstrated, on the one hand, that substances were persistent to a variable degree during the transport along the river segment. On the other hand, transformation processes seemed to be dependent on the time of day. The investigated compounds could be separated into a conservative (e.g. the phosphorous flame retardants TCPP und TDCPP and the pharmaceutical carbamazepine) and a reactive group. The latter comprised substances that were eliminated mainly during daytime (e.g. the disinfectant triclosane und das phosphorous flame retardant TDCP) and others that were transformed as well during nighttime (e.g. the synthetic fragrance HHCB and the pharmaceutical oxcarbazepine). A likely explanation is the variable sensitivity to photodegradation. Next steps will be a more detailed investigation of the processes involved and the factors regulating them. Also toxicological potentials and effects will be further studied.

MO112
Source Apportionment of Trace Contaminants in Urban Sewer Catchments
S. Comber, Plymouth University / Environmental Science; M. Gardner; V. Jones, Atkins Ltd; B. Ellor, UKWIR
Sampling and analysis of Water Framework Directive priority chemicals was undertaken in 9 urban catchments across the UK. Over 9,000 samples were collected from a number of different catchment sources including tap water, domestic waste water, surface water runoff, trade discharges, town centre and light industrial estate wastewaters. Determinands included the main trace metals of interest, PAHs, persistent organic pollutants and a number of common pharmaceuticals, as well as the common wastewater constituents including nutrients, organic carbon, BOD, COD and suspended solids. Loads of the chemicals from each catchment entering the local wastewater treatment works were estimated and were shown to be relatively consistent between different catchments after taking population into account. The study highlighted the importance of domestic wastewater as a source of contaminants, including metals and trace organic substances (such as EDTA, bisphenol A, nonylphenol and TBT). Concentrations in trade discharges were important in some locations in the cases of nonylphenol, EDTA, TBT as well as for some metals such as copper, zinc and nickel. Runoff exhibited significant concentrations of PAHs, lead, and TBT. Contributions to the total load from town centre and light industrial estate sources were generally less than 10% of the total.

MO113
Long-term monitoring of volatile methylsiloxanes (VMS) in aquatic environments impacted by wastewater effluent: experimental design and results from the first three years of collection.
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Boehmer, Evonik; N. Suganuma, K. Kobayashi, Silicone Industry Association of Japan (SIAJ)
Volatile methylsiloxanes (VMS) are widely used in consumer applications. Because wastewater represents the major post-use disposal route for VMS, the Silicone Industry Associations in North America, Europe, and Japan initiated a global monitoring program on VMS in surface sediment and aquatic biota. Aquatic environments that were selected as study areas for the monitoring program were impacted by municipal wastewater effluents and included Lake Pepin (USA), Lake Ontario (on the border between Canada and the USA), Oslofjord (Norway), and Tokyo Bay (Japan). The target objective of the monitoring program was to determine if VMS concentrations were stable or changing, based on detecting with 80% power (β=0.20) a statistically significant (α=0.05) annual rate of change of ±6% per year over a 5 year period (net change of -27% to 34%). Target matrices for each study area include surface sediments, a high trophic level piscivorous fish, and several low trophic level forage fish and benthic invertebrates near the base of the food chain of the predator. The expected minimum project duration of 5 years was estimated from a temporal trend power analysis that was optimized using the within-year variability for VMS concentrations observed during prior monitoring programs conducted on Lake Pepin and Oslofjord. Project duration and experimental design are annually re-evaluated and modified as needed to maintain or improve upon the defined objectives of the project. This presentation will provide an overview of the project and the experimental design for each study area. Available results from the first three years of sample collection and analysis will also be discussed.

MO114
Occurrence and distribution of 12 phenolic compounds in wastewater treatment plant and rivers surrounding a chemical industrial park in China
Q. Xian, Nanjing University; C. Chen, Environment Institute
Abstract: Due to the high toxicity, phenolic compounds were recognized as important pollutants in the environment. Now, phenols were widely used in chemical industry, such as plasticizers, resins, colors, detergents and antioxidants. A GC-MS method of derivatives was developed for determination of 12 phenols in wastewater and surface from a chemical industrial park (CIP) which was located in Yangtze River Delta,East China. Wastewater samples were collected from wastewater treatment plant (WWTP) of the CIP and one chemical company using phenols as raw material in CIP. Surface water samples were collected from the rivers alongside the CIP. Solid phase extraction was used as pre-treatment methods. The total concentrations of phenols in influent and effluent of wastewater treatment station from chemical factory were 1153μg/L and 45.67μg/L. And the effluent was transported into WWTP. In addition, the total concentrations were 366.3μg/L, 254.9μg/L, 81.49μg/L, 56.38μg/L, 122μg/L and 72.47μg/L respectively from the influent, regulation tank, fluidized bed, aeration tank, secondary sedimentation tank and effluent of WWTP. The results showed more than 80% phenols were removed. However, the levels of phenols in secondary sedimentation tank were higher than that in fluidized bed and aeration tank, which implied phenols were formed in secondary sedimentation tank. The total concentrations inChuheRiverlocated in the north of CIP were 41.56μg/L, 24.25μg/L, 20.42μg/L and 18.88μg/L, which showed a decreasing trend from upper to low reach along the CIP. The Yangtze River was located in the south of CIP where there was a drink water source calledJiajiangRiverwith lower concentrations, 3.22μg/L. While the total concentrations were 58.50μg/L, 115.0μg/L, 93.55μg/L, 68.38μg/L, 39.67μg/L and 32.11μg/L at 6 sampling sites from upper to low reach ofYangtze River. This indicated that wastewater containing phenols from CIP and other pollution sources was discharged intoYangtze River, resulting in higher concentration in the middle reach than that in upper and low reach. Overall, the distribution and fate of phenols are important for us to recognize chemicals risk around the CIP, and for government to conduct chemical management of the CIP such as reducing, replacing and recycling. **Keywords:** Phenolic compounds; fate;ChemicalIndustrial Park; GC-MS analysis

MO115
Ozonation or powdered activated carbon: Comparing the ecotoxicity of wastewater after two advanced treatment steps
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For an elimination of micropollutants advanced treatment of wastewater using ozonation or powdered activated carbon is recently being implemented in wastewater treatment plants. Questions have been raised regarding the efficiency of these treatments and the potential toxicity of transformation products. Bioassays are a suitable tool to address these questions. This was demonstrated in the project "Strategy Micropoll" of the Swiss Federal Office for the Environment (FOEN) as well as in a number of other studies. In order to choose the best advanced treatment method at the wastewater treatment plant (WWTP) Basel, two technologies were assessed: (i) ozonation followed by a moving bed reactor (MBR), and (ii) powdered activated carbon addition followed by membrane filtration (PAC). Samples were analyzed for organic micropollutants and ecotoxicological effects before and after treatment. The focus lay on studying the removal efficiency for polar, persistent and bioactive substances as well as possible ozonation by-products. Ecotoxicological effects of the treated and untreated wastewater were assessed using green algae, water flea, amphipods and early life stages of rainbow trout. The estrogenic potential of the wastewater was evaluated using a yeast estrogen screen (YES) assay. It was demonstrated that both ozonation-MBR and PAC treatment are useful measures to reduce the effects of micropollutants in waterbodies. The overall elimination rate (conventional tertiary treatment plus advanced treatment) regarding specific effects (estrogenic activity and inhibition of algae photosynthesis (photosystem II)) was generally above 90%. Overall, advanced treatment led to a reduced toxicity in bioassays, and thus a lowered risk of adverse effects. One assay, the chronic reproduction assay with *Ceriodaphnia dubia*, revealed higher toxicity in ozonated and/or MBR-treated wastewater in two of three measurement campaigns. These results should be verified in further studies. All bioassays applied were generally well suited to assess the performance of the advanced wastewater treatment methods. Both test types, cell-based *(in vitro)*-bioassays as well as tests with whole organisms (*in vivo)* yielded valuable results. The quality of treated effluent was significantly improved ultimately resulting in improved surface water quality. The observed toxicity on *C. dubia* in the ozonation and/or MBR-treatment should be investigated further.

MO116
Optimization of biosorption of copper (II) ions from wastewater on the cone biomass of black pine (Pinus nigra L.)
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Biosorption is a physicochemical process that occurs naturally in certain biomass (biosorbent) which allows it to bind contaminants, in this case metal ions of salt dissolved in water. It is a reversible chemical process resulting from varied affinity of ions for deprotonated biosorbent. It also has significant advantages compared to other processes of water purification: treated water can be reused, and a biosorbent can often be regenerated. The aim of this study was optimization of Cu^{2+} adsorption from aqueous solutions on the cone biomass of black pine (*Pinus nigra L.*), as well as regeneration of biosorbent. Sieved powder of ground mature scales of black pine cones (particle size ≤125 μm) was used as biosorbent. The biosorption equilibrium level was determined as a function of contact time, pH, temperature, and biosorbent concentrations. The amount of Cu^{2+} adsorbedon the biosorbent was defined as the difference in concentration of Cu^{2+} in the solution before and during biosorption. Concentration of remaining (nonadsorbed) Cu^{2+} was determined by atomic absorption spectroscopy. The entire set of performed measurements showed that adsorption of Cu^{2+} from aqueous solutions increased with pH and temperature of the solution. The maximum copper biosorption of 99.27% occurred at 70ºC and pH 7.20. Biosorption equilibrium has been reached in 80 minutes, and the biosorbent concentration for adsorption of 10 mgL^{-1} Cu^{2+} from 0.5 L solution was 800 ppm. Acidic conditions proved to be better for the regeneration of biosorbent compared to alkaline conditions. The best fit to the data was obtained with the Langmuir adsorption isotherm. Our results indicatethat the cone biomass of *Pinus nigra L.* represents a suitable biosorbent for the removal of Cu^{2+} from wastewaters with high adsorption capacity.

MO117
Eco-engineered systems for removal of micro-pollutants from WWTP effluents: technical and scientific recommendations for optimal design of intermediate areas discharges downstream WWTP
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The protection and recovery of aquatic ecosystems, required by the Water Framework Directive, led to consider new refining wastewater treatment processes, to remove nitrogen, phosphorus as well as priority substances. Eco-engineered techniques develop and are likely to be effective in downstream areas in order to remove these substances, if the technique and the design are properly chosen. In France, more than 550 intermediate WWTP discharge in downstream areas were identified in 2011 (IRSTEA, 2012). The feedback on the treatment efficiency of eco-engineered systems and the conditions of removal of individual substance of interest are badly known; yet, the biodiversity potential is often emphasis for these areas. The wide variety of eco-engineered systems and the different architecture designs enable different configurations in intermediate areas: for example the use of one or several different systems, in combination or not. In this context, it is important to understand how they work and to test their performance. For this, a research project is implemented within Veolia Environment Research & Innovation. This project aims to provide technical guidelines for the implementation of eco-engineered systems in intermediate areas of WWTP's

discharge (sizing, order, techniques etc.) and establish performance levels of various techniques by families of substances. First, a compilation of the available knowledge on eco-engineering systems for advanced treatment of WWTP effluent was done, with the aim of assessing removal mechanisms likely to occur in each system (adsorption, photodegradation etc.). In parallel, relevant compounds, regarding their occurrence and ecotoxicity were sought and selected, and knowledge in literature on the removal mechanism of each micropollutant was reported. In a second phase, the project aims to experiment various eco-engineering techniques as tertiary treatment of wastewater in order to check their treatment capacities for the list of substances selected. This step is necessary for a deeper understanding of processes in eco-engineered systems, especially the relevance of the different removal mechanisms and conditions for removal for each individual micropollutant of interest. Finally, rules and design guidelines will be delivered for eco-engineered systems targeting the removal of specific micro-pollutants and their removal rates in each system.

MO118
Degradation of recalcitrant micro-pollutants in designed soil biofilters
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Soil biofilters are efficient to remove micro-pollutants from water so they could be used as a supplementary step in wastewater treatment plants. However, soil biofilters mechanisms are not well understood. Regular soil biofilters can be difficult to control due to sorption to the matrix and formation of water pathways. In contrast, designed soil biofilters are easy to operate because the sorption is controlled; they present an even pore-distribution and are simple to reproduce and up-scale. Two different designed biofilters were studied under aerobic conditions and at ten different flows. The transport and degradation kinetics of typically recalcitrant compounds including pharmaceuticals, fungicides and X-ray contrast has been examined at real wastewater levels in the designed soil biofilters. The transport was studied by controlling the compound's elution with an on-line biofilter-HPLC-UV system. The concentrations of these compounds were off-line analyzed by means of HPLC-MS/MS at the inlet and outlet of the biofilters. The sand biofilter showed to be a good system to study compound degradation because the removal of the compounds was mainly controlled by biodegradation and not sorption. When operating an intermediate flow of 11.8 L m^2h^{-1} the removal efficiencies of the sand biofilter were: 41, 94, 17, 58, 58, 57 and 85% for diclofenac, propranolol, propiconazole, tebuconazole, iopromid, iohexol and iomeprol correspondingly. These results will be compared to a biofilter containing peat to increase the contact time of the compounds in the biofilter. Initial tests revealed higher removal rates in the peat-amended biofilter. For most of the compounds the removal efficiency was contact time dependent and followed first order kinetics, which allowed to calculate removal rates. Propiconazole and tebuconazole did not show any correlation with the contact time.

MO119
Removal of compounds with estrogen-, androgen- and dioxin-like activities by various waste water treatment technologies studied by in vitro bioassays
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Endocrine disruptors and compounds with dioxin-like activities are widely present in wastewater effluents and can cause negative effects in aquatic biota. Wastewater treatment plants (WWTPs) can partially remove these compounds during primary and secondary treatment. However, substantial amounts of these compounds can still remain in treated effluents. Recently, some tertiary treatment steps like ozonation or active carbon filter were installed in some advanced WWTPs to remove micropollutants more effectively. The present study focused on various WWTPs from localities at the river Schussen, a tributary to lake Constance. Dioxin-like activity and also endocrine activities like anti/estrogenicity and anti/androgenicity were measured in effluents of the WWTPs after different treatment (primary or secondary treatment, ozonation, charcoal and sand filter etc.) using different *in vitro* bioassays. Estrogenic activity was relatively high in effluents after primary treatment step (influents), and it was significantly lowered after the secondary treatment. Interestingly, estrogenic activity of the samples collected after the tertiary treatment steps were often similar or slightly higher then effects after secondary treatment step only. Majority of the samples (both influents and effluents) did not show any antiestrogenic potential in our assay. Some of the influents had significant androgenic activity and most of the influents also caused antiandrogenicity. On the other hand, no significant anti/androgenicity was found in the effluents. Significant dioxin-like activity was found only in few of the influents. However, it was high in effluents of most samples after secondary treatment. Effect potentials were again removed (non significant effects) when tertiary treatment was applied. In summary, although the study demonstrated successful removal of majority of bioactive contaminants during different WW treatment steps in situ, some activity in the effluents (especially estrogenicity) can still be of ecotoxicological concern. [Research is part of the project SchussenAktiv*plus* which is funded by the German Federal Ministry for Education and Research (BMBF, funding number: 02WRS1281A) within the RiSKWa programme and cofounded by the Ministry of Environment Baden-Württemberg].

MO120
Effects of differently treated wastewater effluents observed with selected in vivo test systems
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Anthropogenic micro-pollutants are substances that are released into the aquatic environment at very low concentrations mainly by sewage treatment plants. Micro-pollutants are often poorly biodegradable, and their removal through sorption to sewage sludge may be limited by their polarity. Concern is growing that conventional methods of wastewater treatment and purification of drinking water are not sufficient to eliminate these substances. Therefore, the development and large-scale application of advanced wastewater treatment procedures has become an important scientific and political issue in recent years. Some methods, e.g. ozonation, can result in the formation of transformation products that may show similar or even greater toxicity than the parent compound. Our study is part of the research program of the BMBF (Federal Ministry of Education and Research, Germany) funded project TransRisk (Characterization, communication and minimization of risks originating from emerging contaminants and pathogens in the water cycle). The study aims to investigate the potential reduction or enhancement of toxic effects of differently treated wastewater effluents. Investigated advanced treatment methods are: ozonation, membrane bioreactor treatment, activated carbon- and biofiltration. *In vivo* exposure experiments are carried out *in situ* with a flow-through system. This has the advantage to cover peak and average concentrations of micro-pollutants and their transformation products in chronic exposure scenarios. The following *in vivo* test systems are chosen based on previous investigations indicating their sensitivity, and in order to cover different trophic levels and taxonomic groups: the reproduction test with *Daphnia magna*, the sediment-water toxicity test with *Lumbriculus variegatus*, and the growth inhibition test with *Lemna minor*.

MO121
Human pharmaceuticals and industrial chemicals in wastewater effluents – first steps toward a combined risk assessment
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While the environmental risk assessment of human pharmaceuticals and chemicals contained in consumer goods is regulated in different legal frameworks in the European Union, these substances are in principle released together into the aquatic environment via wastewater treatment plants. Hence, the consideration of the potential combined toxicity of these substances may be needed to fully evaluate environmental risks across different regulatory frameworks. In order to develop a concept for this need and experimentally verify its applicability, a number of pharmaceuticals and industrial chemicals were selected to be tested singly and in combination. First results regarding the toxicity of the single substances toward green algae (growth inhibition) and *Daphnia magna* (reproduction) will be presented. The determined effect concentrations will be compared to predicted environmental concentrations (PEC), if available, and measured concentrations in wastewater effluents in order to support a single-substance based risk assessment. Prospectively, this single-substance risk assessment will be compared to a risk assessment taking into account joint effects based on prediction and experimental observation.

MO122
Environmental Risk Assessment of Effluent from wastewater treatment plants along Henares-Jarama-Tajo river basin (Madrid, Spain)
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The presence of pharmaceuticals and personal care products and (PPCPs) in the environment is due to their incomplete elimination in wastewater treatment plants (WWTP). So these substances are found in wastewater treatment plant effluents and surface waters. Even low concentrations of these substances may lead to undesired effects in aquatic systems. The goal of this work is to assess the environmental risk of 49 PPCPs along the Henares-Jarama-Tajo river basin (Madrid, Spain). The concentrations of the PPCPs, measured during four seasonal periods representing the different flow conditions of the river, were compared with PNECwater values to assess the potential risk of sub-lethal effects on aquatic organisms. Twelve

sampling sites were selected up- and downstream of neighbouring cities. Seasonal and spatial variations of the PPCPs in surface waters of the Henares river basin were visualised by transferring the results to a Geographic Information System (1.12.0 gvSIG Association). This study was funded by Spanish projects RTA2010-00004 and CTM2010-19779-C02-01/02

MO123
Fish cell-based biosensor for evaluating water quality
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Cell-based tests play a central role and mark a major trend of the development of alternative assays to animal tests in environmental risk assessment. Millions of fish are used worldwide each year for evaluating water effluent, in the fish acute toxicity tests that take days to complete and use death as the benchmark. In addition to the societal and ethical concerns, the comparative sensitivity of the test is also an issue to be addressed. We have previously shown that the viability-based *in vitro* assay of the rainbow trout (*Oncorhynchus mykiss*) gill cells indicated very good agreement with the *in vivo* fish tests for a wide range of chemicals[1]. This suggests the great potential of adopting the cell system for evaluating water conditions. It therefore serves as the basis for our current project of developing a fish cell-based biosensor in the frame of the Swiss Nanotera project Envirobot (http://www3.unil.ch/wpmu/envirobot/), which aims to build a robotic system for autonomously sampling and analysing water quality. We use the fish gill cells for the bio-recognition of pollutants based on their overall adverse effects on the cells. Cells are cultured on purposely built biochips with embedded electrodes that detect changes, through the measurement of cell impedance, in a variety of parameters including cell growth, migration, morphology, cell-to-matrix and cell-to-cell interactions. The perturbation of the cells by chemical stimuli is read out as changes in the cell impedance, and monitored continuously. Our results of the cell exposure to varying concentrations of the toxicant sodium dedocyl sulphate (SDS) indicates that this system is effective and sensitive in detecting cytotoxicity, even in low doses of SDS which caused no detectable change in microscopy. In addition, conventional end-point viability assays including Alamar Blue, CFDA-AM and Neutral Red that measure vital signs of cellular metabolism, membrane integrity and lysosome integrity, respectively, were also carried out in parallel to the cell-impedance assay. Sensitivity of the cell-impedance based method was comparable or slightly more sensitive to the conventional end-point viability assays, with the advantages of being non-invasive, label-free and monitored in real-time. These results provide the basis for our future steps to test a large scale of chemical pollutants in water without using the whole fish, to optimize the system and integrate it into the Envirobot for field use.

Landscape ecotoxicology and spatially explicit risk assessment of toxicants (P)

MO124
The chemical behavoir of Cu, Zn, Cd and Pb in a eutrophic reservoir: speciation and complexation capacity
A.E. Tonietto, Universidade Federal de São Carlos / Botany; A. Lombardi, Universidade Federal de São Carlos; R.B. Choueri, Universidade Federal de São Paulo; A.A. Vieira, Universidade Federal de São Carlos / Botany; M.G. Melao, Universidade Federal de São Carlos / Hidrobiologia
This research aimed at evaluating metals speciation and interactions with\n natural dissolved organic materials in a eutrophic reservoir. Water\n samples were obtained in dry and rainy seasons, and PCA identified\n seasonal influences of metal input sources into the reservoir. The results\n showed higher labile Zn and Pb in the dry season, but complexed Zn\n dominated during the rainy season. Total dissolved Cu and Pb\n concentrations were higher in the dry season, with the concentration order\n Zn > Cu > Pb > Cd. Higher percentile of Cu and Zn remained complexed with\n the dissolved fraction, while Pb was associated with particulate\n materials. Cd, Cu, Pb and Zn complexation capacity indicated higher ligand\n concentration for Cu, followed in decreasing amounts Pb, Zn and Cd.\n Nevetheless, the strength of association for all metals and the respective\n ligands were similar.

MO125
Pesticides reduce regional biodiversity of stream invertebrates
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The biodiversity crisis is one of the greatest challenges facing humanity, but our understanding of the drivers remains limited. Thus, after decades of studies and regulation efforts, it remains unknown whether to what degree and at what concentrations modern agricultural pesticides cause regional-scale species losses. We analyzed the effects of pesticides on the regional taxa richness of stream invertebrates in Europe (Germany and France) and Australia (southern Victoria). Pesticides caused statistically significant effects on both the species and family richness in both regions, with losses in taxa up to 42% of the recorded taxonomic pools. Furthermore, the effects in Europe were detected at concentrations that current legislation considers environmentally protective. Thus, the current ecological risk assessment of pesticides falls short of protecting biodiversity, and new approaches linking ecology and ecotoxicology are needed. For details see: Beketov M.A., Kefford B.J., Schäfer R.B., Liess M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. PNAS, 110: 11039-11043.

MO126
Ecological community sensitivity – tame the unpredictable or an evidence from a cross-Eurasia experiment
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Ecology has a reputation of science with poor predictive potential. Responses of biological communities to stressors are frequently considered as case-specific and unpredictable. We suggest that predictability depends on the parameter considered, and modulations in integrative parameters such as community sensitivity can be predictable. We performed an experiment with artificial ponds located in Central Europe (Germany) and Northern Asia (Siberia, Russia). The pond zooplankton communities were exposed to an insecticide and their environment was manipulated to alter the community sensitivity. The results showed unprecedented consistency of the community sensitivity modulations between the two regions, with the sensitivity differing by a factor of up to 100. Furthermore, the results suggest that integrative parameters can be predictable, while describing the community reaction in terms nomenclature-based changes lacks generality and predictability. For details see: Beketov M.A., Stampfli N.C., Yurchenko Yu.A., Belevich O.E., Knillmann S., Noskov Yu.A., Liess M., 2013. Ecological community sensitivity – tame the unpredictable or an evidence from a cross-Eurasia experiment. in preparation.

Personal care products in the environment: strengthening science to support regulation (P)

MO127
Comparison of lipid normalized concentrations of cyclic volatile methylsiloxanes in differing lipid matrices of fish collected from marine and freshwater systems
J.A. Durham, Dow Corning Corp / Health and Environmental Sciences; D.E. Powell, R.M. Seston, Dow Corning Corporation / Health Environmental Sciences
To best understand the movement of environmental contaminants through an aquatic food chain, concentrations of these contaminants are determined in whole body homogenates of fish and normalized for lipid content. Due to the necessary equipment to properly homogenize potentially large fish, some researchers and agencies prefer to select individual tissue types for contaminant analysis. Additionally, if the focus of the research is to estimate human exposure, only the edible portions of the fish may be analyzed. The overall objective of this study was to determine if concentrations of cyclic volatile methylsiloxanes (cVMS) in differing lipid matrices would become equivalent once normalized for the lipid content of those matrices. Liver, skin-off fillet, and the remaining carcass of Atlantic cod collected from Oslofjord had concentrations of cVMS that differed, with skin-off fillets having the lowest concentration and liver having the highest concentration when normalized for the lipid content. The results showed a high degree of variability across the 6 fish analyzed with a distinct difference between the liver matrix and skin off fillets when normalized for lipids. Five additional fish species were collected from Lake Champlain Vermont, USA and were analyzed as skin off fillets and remaining carcass. This work also indicated a lower concentration when normalized for lipids of the skin off fillets. Samples were extracted by a standard solvent extraction method and analyzed by GC-MS. This work was supported by Silicones Europe on behalf of the Global Silicones Council.

MO128
Gas phase reaction rates of cyclic volatile methyl siloxanes with the hydroxyl radical as a function of temperature
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Cyclic volatile methyl siloxanes (cVMS) are major constituents of many personal care products that are emitted and partition mainly into air in the environment. In ambient air, the main degradation pathway of cVMS is likely to be reaction with the hydroxyl radical (˙OH). Because cVMS partition mainly into air, this degradation rate largely determines their persistence in the environment. We measured rate constants for the gas-phase reaction of several cVMS with ˙OH using the relative rate technique at temperatures between 313 and 363K, using an experimental design based on the work of Anderson and Hites (1996). The reactions were carried out in a 195 ml reaction chamber with continuous monitoring of the concentrations of cVMS and reference substances by a mass spectrometer operating in electron ionization mode. ˙OH was generated *in-situ* in the reactor from ozone by irradiation with ultraviolet light in the presence of water vapor. These are the first measurements reported for the reaction rates of the cVMS species D_6 and D_7 with ˙OH, and the first temperature-dependent rate constants reported for the cVMS species D_3, D_4 and D_5. The Arrhenius expressions can be extrapolated to values at 297K that agree well with earlier measurements conducted by Atkinson (1991). The Arrhenius expression estimated for the reaction of D_5 with ˙OH is: ln $k = -22.7 \pm 1.3 - 9500 \pm 3600 / (R \cdot T)$ Where k is the second-order reaction rate constant [$cm^3 molec^{-1} sec^{-1}$]. The Arrhenius expressions for the other cVMS are reported on the poster. The activation energies for reactions with ˙OH are similar for all the cVMS studied. The relative reaction rate between two cVMS is therefore nearly independent of temperature. Anderson, P. N.; Hites, R. A., *ES&T* **1996,** *30* (1), 301-306. Atkinson, R., *ES&T* **1991,** *25* (5), 863-866.

MO129
Hydrolysis and Volatilization of Linear Volatile Methylsiloxanes in Soil
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Linear volatile methylsiloxanes (LVMS) such as hexamethyldisiloxane (L2), octamethyltrisiloxane (L3) and decamethyltetrasiloxane (L4) are a group of low-molecular-weight silicone fluids primarily found as an impurity in silicone products and personal care products. The objective of this study was to determine the effect of their molecular structure on their hydrolysis and volatilization rates in soil. In a previous project, the hydrolysis and volatilization rates for L3 in two soils have been measured, including the Londo soil from Michigan. In the current study, the hydrolysis and volatilization rates of ^{14}C-labeled L2 and L4 were measured in the same Londo soil at four moisture levels: 32 % RH, 42%RH, 92%RH and 100% RH. In closed systems, the recoveries of the LVMS radioactivity under various moisture levels (including 100% RH) were close to 100%. At 22.5 °C, the hydrolysis half-lives of L2 and L4 varied from 1.4 days for L2 at 32%RH to 107 days for L4 at 100%RH. In open system at the same temperature under 100%RH, volatilization rates varied from 3 hours for L2 to 4 days for L4. Combined with the data for L3 from the previous study, the hydrolysis half-lives (τ_h in days) for all three LVMS at 22.5 °C was found to be related exponentially to the number of D (dimethylsiloxane) units (N_D): log $\tau_h = 0.36\ N_D + 0.88$ ($r^2 = 0.993$). Similarly, the volatilization half-lives (τ_v in days) of three lVMS in open systems at 100%RH was also related to N_D exponentially: log $\tau_h = 0.74\ N_D - 0.92$ ($r^2 = 0.991$). Based on those data, hydrolysis of LVMS should be the predominant removal mechanism in dry soil, while volatilization should be predominant in wet soil. Nevertheless, LVMS, once released to soil, should not be expected to remain long in soil under either dry or wet conditions

MO130
Organic carbon/water partition ratios (Koc) of cyclic volatile methylsiloxanes
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The environmental fate of cyclic volatile methylsiloxanes (cVMSs) has recently attracted the attention of environmental chemists and regulators due to their large production volumes, their persistence, and their potential for bioaccumulation [1]. cVMSs degrade within days or weeks when they are released to the atmosphere [2], but their residence time in surface waters is substantially longer [3]. When cVMSs are released to surface waters they are sorbed to suspended particles coming out of wastewater treatment plants and eventually fall to the sediment. From the sediment, cVMSs can move back to the water column and from there volatilize to air or undergo hydrolysis. Their release from the sediment depends on i) the amount and the quality of the organic carbon present and ii) their partitioning between organic carbon and water [4]. We studied this behaviour in a series of laboratory experiments and interpreted it with mathematical modelling. In the experiments various amounts of sediment were spiked with cVMSs and immersed in a volume of water. The water was continuously stirred and the headspace was purged by a nitrogen stream, which carried the cVMSs to an ENV+ column. The amount of the chemicals in the ENV+ was measured at various time points over the course of the experiment (72 hours). Aside from cVMSs, the sediments were also spiked with dichlorobenzene and polychlorinated biphenyls, which were used as benchmarking chemicals for the water-to-air and sediment-to-water mass transfer coefficients. The data for the benchmarking chemicals acquired from the experiment were used to calibrate a fugacity-based multimedia model, which in turn was used to estimate the organic carbon/water partition ratios (KOC) of cVMSs. We compared and contrasted the Koc values for the CVMSs derived from our experiment with other measurements [4] and QSAR models. References [1] Environment Canada, 2008. Registry Number 541-02-6. [2] Atkinson R. 1991. Environ Sci Technol. 25 (5) 863-866. [3] Durham J. 2006. Silicones Environment, Health and Safety Council. Study Number 10040-102. [4] Whelan MJ, Sanders D, van Egmond R. 2009. Chemosphere. 74 (8) 1111-1116.

MO131
Understanding the fate and bioaccumulation of cyclic volatile methyl siloxanes in Arctic lakes
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Cyclic volatile methyl siloxanes (cVMS) are used in personal care products and are frequently found in the environment. There is an ongoing scientific debate regarding cVMS bioaccumulation potential in aquatic organisms with contradicting results from recently reported studies. In this study we describe a holistic investigation of the environmental behavior of cVMS in lake Storvatn (70 °N, 23 °E), an Arctic lake in northern Norway that receives wastewater emissions. Existing and newly developed methods using headspace gas chromatography are applied to analyze the occurrence of cVMS in water and sediment, while established liquid extraction methods are used to analyze cVMS in biota from the lake. The measurements are used in combination with models to facilitate a mechanistic understanding of the environmental behavior of cVMS in the system. More specifically, a dynamic multimedia environmental fate model, parameterized for the physical and chemical characteristics of the lake, will be used to drive a bioaccumulation model. Preliminary simulation results indicate that concentrations of cVMS in the water column will increase during winter, which highlights the importance of seasonal characteristics such as low temperature and ice cover in these kinds of systems.

MO132
ANALYSIS OF UV STABILIZERS IN LIQUID AND SOLID TREATMENT PLANTS PRODUCTS AND THEIR FATE IN ENVIRONMENTAL SAMPLES FROM GRAN CANARIA ISLAND (SPAIN)
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Within the wide range of Personal Care Products (PCPs), the UV filters are a group of compounds which use have notedly increased in the last decades. Due to the growing concern about the link between sunlight exposure and skin cancer, they are added not only in sunscreem but also in variety of cosmetic such as lip gloss, shampoos, hair dyes, makeup, etc., to reflect and absorb the solar radiation. After be used, these compounds can reach the environment through recreational activities such as swimming and bathing in oceans, lakes or rivers (direct inputs) [1] or after passing throughout wastewater treatment plants without be removed. One type of the most used UV filters is the Benzotriazole UV stabilizers (BUVSs) family, compounds with a phenolic group attached to a heterocyclic structure with three N atoms. It has been demonstrated that their derivatives can present negative effects over aquatic systems. For example they are mutagenic in bacterial systems and toxic in plants and can exert adverse effects on the fecundity and reproduction of fish [2]. We evaluated, for first time, the presence of seven BUVSs in liquid and solid samples from Gran Canaria Island (Spain) using on-line solid-phase extraction (On-line SPE) for liquid samples and microwave-assisted extraction followed by On-line SPE (MAE-On-line SPE) for solid samples, both coupled to ultra-performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS). The sensible optimized methodologies provides LODs between 0.73-4.18 $ng \cdot L^{-1}$ and 53.3 to 146 $ng \cdot kg^{-1}$ for liquid and solid samples, respectively, and they allows the detection of some BUVSs in the different products from several treatment plants and environmental samples taken in Gran Canaria Island (seawater collected in different points of the coast, sand of different beaches and marine sediments collected near to a wastewater submarine outfall). References: [1] M.E. Balmer, H.R. Buser, M.D. Müller, T. Poiger, Environ. Sci. Technol. 39 (2005) 953-962. [2] S. Montesdeoca Esponda, T. Vega Morales, Z. Sosa Ferrera, J.J Santana Rodríguez. TrAC-Trends Anal. Chem. 51 (2013) 23-32.

MO133
Occurrence of synthetic musks in mainland Portugal – a biosampling approach using pine needles
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Vegetation has been used as a passive biosampler to evaluate atmospheric pollution. In particular, pine trees are commonly chosen due to their widespread occurrence and evergreen leafs (needles). Pine needles morphology presents an

outer waxy cuticle, which offers a remarkable capacity to entrap compounds like semi-volatile organic pollutants. Our work team has earned experience in the use of this kind of matrix, studying several of these contaminants, namely PAHs, pesticides and flame retardants, among others. Concern about synthetic musks has been rising in recent years. Their common use as fragrance fixatives in scented products allied to their lipophilicity, persistence and potential biological effects may pose a risk to ecosystems. Although several studies have been published regarding their occurrence in water, sludge and fauna, information about atmospheric levels is still scarce. As opposed to active or other types of passive air sampling, employed in those studies, biomonitoring using pine needles avoids a previous sampling site set-up and may act a "biological data loggers" of pollution as needles remain in the tree for several years. To the author's best knowledge, this is the first study employing vegetation for the detection of synthetic musks. An analytical method employing ultrasound assisted extraction, followed by solid-phase extraction and gel permeation chromatography clean-up was previously developed and validated, allowing the assessment of levels of 5 nitromusks and 6 polycyclic musks. Needles of the two most common pine species in Portugal (*Pinus pinaster* and *Pinus pinea*) were collected at 30 sites of different exposure pattern types. Cashmeran and galaxolide were the most commonly detected musks. The mean total concentrations ranged between < 1 ng/g and 460 ng/g. Pine needles proved to be suitable for the monitoring of the atmospheric presence of musks. Acknowledgements The authors wish to thank Fundação para a Ciência e a Tecnologia (FCT - Portugal) for the Projects PTDC/AGR-CFL/102597/2008 and EXPL/AAG-MAA/0981/2013 and grant SFRH/BPD/76974/2011. This work has been partially funded by the European Union Seventh Framework Programme-Marie Curie COFUND (FP7/2007-2013) under UMU Incoming Mobility Programme ACTion (U-IMPACT) Grant Agreement 267143.

MO135

Polycyclic musks whistle-blowing in the environment: appraisal of anthropogenic impact on fresh water systems

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With a multi-tracer approach including different physical-chemical parameters, sum parameters, nutrients, and two polycyclic musk compounds (PMCs: galaxolide and tonalide) the small river Wetter in Hesse, Germany was investigated from its source to mouth (64 km length). By this, the impact of land-use on its water quality was assessed by involving 20 sampling points at 4 sampling campaigns. Within this catchment area of 516 km^2 more than 50% are used by highly productive agriculture, approx. 35% are forested land, whereas ca. 12% are settlement area. As an approximation, population equivalents which are attributed to the connected sewage treatment plants, are used to assess the impact of municipal waste water on the river Wetter. Discharge values at several control points were used to calculate loads of nutrients and PMCs. Galaxolide and tonalide were determined by a head-space solid phase microextraction method which enables to discriminate between total and freely dissolved amounts of these compounds. Precision of this highly efficient and sensitive method was confirmed by exemplary standard addition measurements. This techniques allows high sample throughput which is indispensible for such analyses on a landscape scale. Nutrients were introduced by diffuse sources from agricultural land-use and municipalities and only phosphate and nitrite could slightly indicate impact of different land-use by increasing concentrations downstream. Mean concentrations of galaxolide and tonalide were, depending on the impact of sewage treatment plants, increased up to 243 ng L^{-1} and 83 ng L^{-1}, respectively. By the calculation of PMC loads, impact of population on the water quality could be explained with a coefficient of determination of 0.98. This resulted in daily loads per population equivalent of 0.19 mg and 0.08 mg for galaxolide and tonalide, respectively. With this study, the appropriateness of both galaxolide and tonalide as reliable indicators for the impact of municipal waste water could be proven. Sediment samples for PMC residues are in progress.

Key-words: tracer analysis, galaxolide, tonalide, land-use

MO136

An evaluation of the ecotoxicological effects and risks of the biocide benzalkonium chloride on freshwater ecosystems

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Benzalkonium chloride (BKC) is a quaternary ammonium biocide widely used in personal care products, hospitals, wood and food industries (e.g. aquaculture). The potential environmental risks of BKC to aquatic ecosystems have been poorly investigated. The main goal of this study was to assess the toxicity of BKC to aquatic organisms through a battery of toxicity tests that allowed a preliminary risk assessment of BKC to four different trophic levels of aquatic ecosystems. Toxicity tests were performed for decomposers, primary producers, primary consumers, and secondary consumers. The results from the toxicity tests were used to build Species Sensitivity Distributions (SSD) and to calculate the BKC median Hazardous Concentrations (HC5% and 50%), separately to each trophic level. Moreover, using Measured Environmental Concentrations (MEC) of BKC, the Potentially Affected Fractions of species (PAFs) were estimated for four different scenarios (raw and wastewater treatment plants effluents and surface water) by joining SSDs and distributions of MEC. Among primary producers, *Pseudokirchneriella subcapitata* was found to be the most sensitive (72h-EC50=69 μg/l) species, followed by *Chlorella vulgaris* (72h-EC50=94 μg/l) and *Lemna minor* (168h-EC50=1340 μg/l). Primary consumers were highly sensitive to BKC (*Daphnia magna*: 48h-EC50=52 μg/l and *Tamnocephalus platyurus*: 24h-EC50=84 μg/l), whereas fish species showed to be slightly sensitive (*Oreochromis niloticus* embryos: 96h-LC50=370 μg/l, *Danio rerio* adults: 96h-LC50=2350 μg/l and embryos: 2060 μg/l). The SSD analysis indicated that primary consumers (HC5=10.8 μg/l) and primary producers (HC5=11.4 μg/l) are the most sensitive groups, followed by decomposers (HC5=152 μg/l) and secondary consumers (HC5=217 μg/l). Due to the high BKC concentrations measured in hospital, laundry and industrial effluents, the PAFs values obtained were > 5% for the four trophic levels studied, suggesting high toxicity of these effluent to aquatic biota. Even after treatment, monitored BKC concentrations in sewage treatment plant effluents and surface water are high enough to exert toxic effects on primary producers and consumers (PAFs > 5%). The results of this study show that there is a need to regulate the use of BKC, to increase the removal efficacy of treatment plants for disinfectants such as BKC, and to include BKC as part of chemical and biological monitoring studies in aquatic ecosystems.

MO137

Chronic toxicity of azo and anthracenedione dyes to embryo-larval fathead minnow

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Synthetic dyes are extensively used in many fields, including paper production, leather tanning, food colouring, personal care products (e.g., hair colour, deodorant, etc…), as well as in textiles and paints. We assessed several azo and anthracenedione dyes from Canada's Chemicals Management Plan (CMP) to determine sublethal toxicity in embryo-larval fathead minnows. Fathead minnow newly fertilized embryos were exposed through the egg stage (5 days) up until 14 days post-hatch, with dye solutions renewed daily. The anthracenedione dyes Acid Blue 80 (AB80) and Acid Blue 129 (AB129) were both non-toxic at the highest measured concentrations tested of 7,700 and 6,700 μg/L, respectively. Both azo dyes Disperse Yellow 7 (DY7) and Sudan Red G (SRG) were highly toxic to larval fish after 20-day exposures to concentrations in the μg/L range. LC50s (based on measured concentrations of dyes in fish exposure water) were 25.4 μg/L (95% CI 24.5-26.4 μg/L) for Disperse Yellow 7 and 16.7 μg/L (95% CI 15.6-17.8 μg/L) for Sudan Red G. Exposure to both azo dyes caused a delayed toxicity response, with larval fish succumbing 4-5 days after hatch. This is important for fish bioassay exposure times and endpoints, as if these dye exposures were ended at the embryo stage or just after hatch, the toxicity of these two dyes would be greatly underestimated. This LC50 fathead minnow embryo-larval toxicity data for the azo dyes is much lower than previously reported in a literature review of dyes where LC50s were generally over 1 mg/L. The predicted environmental concentration for these dyes in the literature review was 20 μg/L, which is similar to the concentrations causing toxicity in our exposures of fathead minnow larvae. Although the two azo dyes were quite toxic in our tests, the concentrations of dyes in the Canadian environment were very much lower than those that caused effects in the current tests. In a total of 100 samples of municipal wastewater effluent from across Canada assessed for these dyes, all were below detection limits (detection limits: 0.122 μg/L (DY7); 0.0389 μg/L (SRG); 1.52 μg/L (AB129); and 33.4 μg/L (AB80)). The similarities of the structures and toxicity responses for the two azo and two anthracenedione dyes in this study support the use of read-across data for risk assessment of these classes of compounds.

MO138

Gene expression analysis of Mysid Crustacea exposed to Triclosan and Triclocarban using DNA microarray

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Triclosan (TCS) and Trichlorocarban (TCC) are widely used as antibacterial agent in various industrial products, such as textile goods, soap, shampoo, liquid toothpaste and cosmetics, and often detected in wastewater effluent. The aim of this study was to investigate toxicologically significant effects of TCS and TCC exposure on gene expression in mysid (*Americamysis bahia*) using DNA microarray. Juvenile mysid were exposed to 0.5 µg/L of TCS and 0.05 µg/L of TCC for 24 hours, and mRNA expression profiles in their whole bodies were analyzed.

We identified statistically significant (p 3) in 312 genes responding to 0.5 µg/L of TCS and 264 genes responding to 0.05 µg/L of TCC. There were merely 231 genes found responding to both exposures of TCS and TCC. The up-regulation in response to TCS and TCC was observed in vitellogenin and clottable protein. These genes are expressed more than 30 times in both experimental plots, which indicates the applicability of them in serving as biomarkers of antibacterial agents in mysid. These results suggested that endocrine disrupting effect of TCS and TCC would be potent for marine crustacean.

MO139

Upregulation of hormonal signaling genes in insect embryos and larvae after exposure to UV filters

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Organic UV filters are emerging aquatic contaminants due to the extensive use in a large variety of products. There is an increasing evidence indicating that several UV filters might have endocrine disruptive effects. Numerous studies have evaluated hormonal effects in vertebrates, mainly reporting estrogenic and androgenic activities in mammals and fishes. Nevertheless, there is still scarce information of hormonal activity in invertebrates endocrine systems. In this work, the effects of UV filters were investigated in different developmental stages of *Chironomus riparius* (Diptera), a reference organism in aquatic toxicology. Expression levels of the gene coding for the ecdysone receptor (*EcR*) and the heat shock gene *Hsp70* were quantified by Real Time PCR following short exposures to five frequently used UV filters: octyl-p-methoxycinnamate (OMC) also called 2-ethylhexyl-4-methoxycinnamate (EHMC); 4-methylbenzylidene camphor (4-MBC); 4-hidroxybenzophenone (4-HB); octocrylene (OC); and octyldimethyl-p-aminobenzoate (OD-PABA). Tje results revealed that all the UV filters tested are able to elicit a fast activation of the ecdysone receptor, a key transcription factor for the ecdysone-genomic response in arthropods. Embryos seem to be much more sensitive to these compounds. Similarly, exposure to UV filters triggered the activation fo the *Hsp70* gene increasing mRNA levels in embryos and larvae. The capability of UV filters to stimulate the expression of hormonal signaling genes that are essential both for larval and embryonic development suggests the possibility of a broad and long-term effect on invertebrate endocrine pathway. These findings strengthen the need for further research about the ecotoxicological implications of chronic exposure to these compounds in aquatic invertebrates.

MO140

Effect of mosquito repellents on aquatic non-target organism: Vertical distribution and predator avoidance in Daphnia

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DEET (CAS 134-62-3), Icaridine (CAS 119515-38-7) and EBAAP (CAS 52304-36-6) are the most common active ingredients in insect repellents, being mainly used against mosquitoes. Application of these repellents to human skin and subsequent wash off leads to environmentally relevant concentrations of these compounds in surface waters. Here we hypothesize that input of these repellents into the epilimnion of stratified lakes might affect the non-target organism Daphnia and lead to (i) avoidance of the upper strata of the lake by Daphnia, and (ii) interfere with the chemically mediated avoidance of Daphnia of its major predator fish. We test this using an indoor bioassay system in which we monitor the vertical distribution of Daphnia in a stratified water column in response to DEET, EBAAP and Icaridine in concentrations ranging of from ng/L to μg/L.

MO141

Analysis of Personal Care Products in Human Placental Tissue

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Last decade, ingredients in personal care products (PCP) have been described as chemicals of increasing environmental concern because of their toxicity, persistence, bioaccumulation, and ubiquity. PCPs are produced in extremely large quantities, thousands of tons per year. Parabens (PB) and UV filters (UV-F) are chemicals widely used as preservatives and sun blocking agents, respectively by personal care products, pharmaceutical and food industries. Many studies show the tendency of UV-F to bioaccumulate in living organisms due to their lipophilicity and stability versus biotic degradation [1, 2]. The ubiquity of these compounds in the environment and even human fluids [3-5] makes necessary further studies to fill the current knowledge gap. This study aims to provide evidence of the bioaccumulation of UV-F and PB in humans. The work describes the development of a new analytical method for multiclass determination of selected UV-F and PB based on high-performance liquid chromatography-quadrupole-linear ion trap-tandem mass spectrometry (HPLC-QqLIT-MS/MS) in women placental tissue. Target compounds were selected by their endocrine disruption activity and frequency of detection in the environment, i.e. benzophenone-1 (BP1), benzophenone-2 (BP2), benzophenone-3 (BP3), benzophenone-4 (BP4), 4-hydroxibenzophenone (4HB), methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB), and butylparaben (BPB). The method involves a liquid-liquid extraction step prior to the HPLC-MS/MS analysis. Detection was carried out by MS/MS under electrospray ionization in negative mode (ESI-), and selective reactions monitoring (SRM). The LC-mobile phase was MeOH and H_2O, both with ammonium acetate 5 mM. The quality parameters of the method along with the suitability of its application to real samples will be presented. **References** 1. Kim J.W., Ramaswamy B.R., Chang K.H., Isobe T., Tanabe S. J. Chromatogr. A 1218 (2011) 3511. 2. Fent K., Zenker A. and Rapp M., Environ. Pollut., 158 (2010) 1817. 3. Gago-Ferrero P., Díaz-Cruz M.S., Barceló D. Anal. Bioanal. Chem. 400 (2011) 2195. 4. Gago-Ferrero P., Díaz-Cruz M.S., Barceló D. J. Chromatogr. A 1286 (2013) 93. 5. León Z., Chisvert A., Tarazona I. and Salvador A., Anal. Bioanal. Chem., 398 (2010) 831. *Acknowledgements:* The authors are members of the Consolidated Research Water and Soil Quality Group of the Generalitat of Catalonia, Spain, (2009-SGR-965).

MO142

IFRA ENVIRONMENTAL STANDARDS: RISK AND HAZARD ASSESSMENT UPDATE FOR 2014

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To assure safety of fragrance ingredients in consumer products, International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environment Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the IFRA Environmental Standards, RIFM reports the most recent results of these additional tests (for both risk and hazard assessments) at both the annual SETAC NA and Europe meetings. These studies include persistence testing (ready biodegradation tests and die-away studies)), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented.

MO143

Analysis and ecotoxicological investigations of Poorly Soluble Cosmetic Compounds – an approach for a reliable environmental risk assessment (ECOSM)

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Personal Care Products (PCP) - often referred to as cosmetics - include a broad range of compounds that are poorly soluble in water, i.e. below 1 mg/L. Due to the high production volumes of PCP, and their typical 'rinse off' application, substantial amounts of these poorly soluble chemicals end up in waste-water treatment plants and may subsequently enter river systems with the effluent. This causes the need for a reliable assessment of their environmental behavior and toxicity to organisms, as requested by the European REACH-Regulation. Due to their high lipophilicity, resulting in extensive adsorption to surfaces like test vessels and organisms, standard ecotoxicity tests are not suitable for poorly soluble substances. The lack of consistent, reliable results due to the difficulties in maintaining constant test concentrations may lead to improper assessment of possible environmental risks. A promising approach addressing this problem is the so called 'poorly solubles approach' making use of an 'ecotoxicological threshold concentration of no concern' (ETNCaqua) for inert substances with a narcotic mode of action. Substances with solubility below this threshold concentration, are expected to have neither acute nor long-term adverse effects on aquatic organisms. The aim of the project ECOSM (ECOtoxicity investigations of COSMetic ingredients) is to develop pragmatic tools to test the ETNCaqua hypothesis in order to allow better environmental risk assessment of poorly soluble substances. At this stage of the project the surfactant precursor Dodecylbenzene has been selected as a suitable model compound. Different techniques for chemical analysis using gas chromatography have been developed successfully. These allow the detection of substance concentrations at the solubility threshold. Using these techniques the

maximum solubility of Dodecylbenzene in different media was exactly determined. Additionally passive dosing techniques have been established to maintain constant substance concentrations at the solubility threshold throughout the duration of various existing ecotoxicity tests with organisms of different trophic levels as proposed by OECD guidelines (e.g. algae, daphnia, fish eggs, and fish). Further methods to reach constant exposure conditions are under development and first ecotoxicity tests have been carried out. The adaptation of the test guidelines to these methods will be discussed.

MO144

How to focus experimental tests only on priority Personal Care Products

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Several ingredients in Personal Care Products (PCPs) are now of recognized increasing environmental concern for their distribution, persistency, bioaccumulation and toxicity (PBT behavior). These ingredients have highly heterogeneous chemical structures and very different properties, for the majority of them persistence and toxicity data are lacking and the environmental behavior is unknown. Moreover, the determination of all the dangerous properties, required by REACH and Cos-me-tics Directive (Council Directive 76/768/EEC), is a long and difficult task. Due to the high variety of these chemicals and the big number of end-points that should be studied it is very important to have tools able to highlight the most dangerous compounds, diminishing the experiments because they could be focused only on the selected compounds. In this study, we propose tools for prioritizing the most dangerous ingredients in PCPs, thus reducing the costs and the animal tests as required by the new Regulations. A big number of chemicals (more than 500), such as flavor and fragrance agents, parabens, phthalates and UV filters have been screened for their cumulative PBT behavior, their acute toxicity on *Pimephales promelas* and their soil sorption on organic carbon. This screening has been done by the corresponding models (PBT-Index, *P.promelas* tox, and Koc) implemented in the software QSARINS and in the new module QSARINS-Chem, using also some chemometric method as Principal Component Analysis (PCA), MultiCriteria Decision Making (MCDM). The results of this screening/ranking study are a valid help for the understanding of the environmental behavior of hundreds of PCP ingredients, belonging to various chemical classes and for the reduction and focus of experimental tests.

MO145

QSAR study of Fragrance Biodegradability for safer alternatives

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Fragrance materials are a group of structurally heterogeneous compounds which have been widely used as ingredients in many consumer and personal care products. Their environmental occurrence, mainly in air and water compartments, is of potential concern for both humans and wildlife. After entering into the enviroment, fragrances may persist, and bioaccumulate into aquatic organisms, likely causing toxicity and endocrine disruption. Biodegradability is a basic information for the evaluation of environmental fate and persistence carried out in human and environmental risk assessment. Predictive approaches, such as those based on quantitative structure-activity relationships (QSARs), can be used to predict biodegradability of untested fragrances as well as support experimental data in a weight of evidence approach. The objective of this work is to propose valid QSAR models for the prediction of ready biodegradability that are specifically applicable to fragrance material. QSAR classification models were developed based on ready biodegradability data measured for a heterogeneous set of 189 fragrances, including nitro-, polycyclic and macrocyclic musks, cinnamates, terpenes, and linalool derivatives. Two different classification methods, namely classification and regression tree (CART) and k-Nearest Neighbors (k-NN), were applied to perform the modelling. Models were validated for their robustness and external predictivity, and the structural applicability domain was verified. A comparison was carried out between the here presented QSARs and U.S. EPA - BIOWIN models, showing a general higher specificity of our QSAR models in recognizing not ready biodegradable fragrances. In conclusion, robust and predictive QSAR models for the prediction of ready biodegradability of fragrances are presented in this study. These QSARs are based on transparent algorithms and on descriptors that are either commercially available or freely calculable, which guarantees a wider applicability and reproducibility of the models. The proposed models can be applied for the screening of fragrance chemicals, even before their synthesis, to support experimental data and to design safer alternatives that are less persistent than existing ones, as also required by REACH Regulation.

Fate and effects of nanoparticles under environmentally realistic conditions (P)

MO146

Impacts of differing nanomaterial surface chemistry on Daphnia magna

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In order to create nanomaterials that are sustainable with little potential environmental impact the mechanisms that govern nanomaterial toxicity need to be understood. Yet there are still questions as to the properties of nanomaterials that lead to toxicity. Variations in nanomaterial surface chemistry may influence nanoparticle toxicity by changing nanoparticle interactions with organisms and directly contributing to toxicity. In this study, the impacts of differentially functionalized gold and diamond nanoparticles on mortality, reproduction, and body size in the toxicological model species, *Daphnia magna,* were compared over acute and chronic exposures. Acute and chronic assays show that negatively charged gold nanoparticles are orders of magnitude less toxic than the positively charged gold nanoparticles. Certain ligands used in nanoparticle functionalization can directly contribute to and increase toxicity of nanomaterials. We also show that nanomaterials can increase the toxicity of ligands by increasing their delivery to the organism and therefore localizing ligand concentration and exposure. In addition, similarly functionalized diamond and gold nanoparticles have differing toxicities in Daphnia which indicates that toxicity is not solely due to surface charge but also dependent upon other properties such as ligand density, structure or particle stability. These results demonstrate that specific nanomaterial properties have great implications for determining nanomaterial toxicity and need to be considered in order to design sustainable nanomaterials.

MO147

Toxicity effects of silver nanoparticles synthetized using Arbutus unedo leaf extracts to the cladoceran Daphnia magna

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Silver nanoparticles (AgNP) are used worldwide and can be found in many types of products, such as domestic disinfectants, cleaning products and even in pharmaceuticals, due to their bactericidal properties. This high use will inevitably lead to an increase release and high impact to the environment. For the past years silver nanoparticles have been synthesized using several physical and chemical methods. Biological and eco-friendly green synthesis processes have also been applied using different plant extracts, like leafs, to reduce metal ions to nanoparticles. This study aimed at comparing the toxicity of AgNP synthesized using different proportions of *Arbutus unedo* leaf extracts on the survival, reproduction and feeding rates of the cladorecan *Daphnia magna*. Toxicity was evaluated through acute and chronic tests (48h and 21 days exposure, respectively) in order to evaluate effects on survival and reproductive output. Feeding rates were evaluated through 24h feeding inhibition tests. Results revealed higher toxicity for AgNP synthesized with a lower proportion of leaf extract, which may be a consequence of a higher release of silver ions compared to the AgNP synthesized with a higher proportion. Using also time as a key variable, and looking at NPs storage time, although acute toxicity levels between both AgNP become similar with time, chronic toxicity continues to show differences between AgNP, where lower proportions of leaf extract continues to induce higher toxicities.

MO148

Toxicity of silver and gold nanoparticles, produced in the gas phase, to the aquatic plant Lemna minor

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The increasing use and applications of engineered nanoparticles (ENPs) in consumer products has raised many questions with regard to their potential toxic behavior. Although an increasing number of studies have focused on the toxicity of ENPs, little information is available on their toxic effects on aquatic plants. This study assesses the effects of silver (AgNPs) and gold nanoparticles (AuNPs) on *Lemna minor* under modified ISO 2079 test conditions. The nanoparticles used in these tests were synthesized using an innovative aerosol-based method (namely the spark discharge generator), which yields nanoparticles of very high purity and provides good control over their size, morphology and composition. Spherical nanoparticles having diameters in the range from 20 to 80 nm were produced in high purity Ar gas and inserted in deionised water. Tested concentrations of AgNPs and AuNPs were 0, 5, 10, 20 and 40 μg L^{-1}. After 7 days exposure to a 10 μg L^{-1} solution of AgNPs *Lemna* cultures showed a statistically significant inhibition of frond numbers ($p < 0.05$). Growth inhibition by up to 90% was observed for all tested concentrations. A 7-day exposure of *Lemna* to the lowest concentration of AgNPs (5 μg L^{-1}) also caused a significant reduction in its relative growth rate (RGR). AuNPs on the other hand, had a positive effect on frond numbers when their concentration was 5 and 10 μg L^{-1}. For these concentrations the relative growth inhibition was 70 and 50%, respectively. At higher concentrations (i.e., 20 and 40 μg L^{-1}) AuNPs exhibited a statistically significant inhibitory effect on frond number ($p < 0.05$). Compared to Ag, AuNPs also enhanced root length and dry weight at 5 μg L^{-1}. Evidently, high-purity AgNPs and AuNPs prepared with the same method have different effects on *Lemna minor* growth. AgNPs were found to be toxic to *Lemna minor* at the lowest tested concentration of 5 μg L^{-1}, whereas AuNPs exhibited a positive effect for some growth parameters tested. This is of particular importance considering the increasing production and use of silver and gold nanoparticles in consumer products, which will render these toxic concentrations more environmentally relevant in the future.

MO149

Fate and effects of 107Ag nanoparticles in Daphnia magna under realistic environmental test conditions

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At present there is a worldwide focus on engineered nanoparicles (ENPs) in the environment, the possible risks they pose and the potential need for nanospecific environmental risk assessment and legislation. The ENPERA project (Engineered nanoparticles interactions with the environment: towards and understanding of the risk they pose) specifically targets engineered nanoparticles and how they influence "system and process understanding" and "what are the effects of distribution of NPs and how can these be dealt with". The project seeks to establish a knowledgebase that will inform the process of understanding the risks posed to the environment from ENPs, specifically focusing on environmentally relevant exposure scenarios and consideration of toxicity and fate of ENPs in the environment and within target organisms. This poster presents the evaluation of the fate and effects of a stable isotope silver particle (^{107}AgNP) in the standardised test organism *Daphnia magna*. Silver nitrate was and used as a comparative "bulk" silver for all experiments. Both short term acute and longer term chronic exposures were conducted followed by an examination of the uptake and depuration within the test organism. As well as assessing a more realistic exposure (e.g. presence of food, natural organic matter (NOM)) the aim was to allow the work to feed into the regulatory process by employing standardised methods (e.g. OECD). The acute toxicity of $AgNO_3$ was greater than ^{107}AgNPs in all experiments. In addition differences in the behaviour of the ENP were observed compared to $AgNO_3$ when assayed with varying environmental parameters e.g. in the presence of food the toxicity of the NPs was reduced but the toxicity of $AgNO_3$ was observed to increase. Additionally the uptake of AgNP was greater than $AgNO_3$ with increasing concentration, indicating a possible nano specific mechanism for uptake of silver. The data was then compared with existing data to asess the suitability of standard methods and compare the toxicity of these NPs with NPs of similar size and composition in the literature.

MO150

Engineered nanoparticle fate model – simulating realistic conditions in a complex natural river system

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An increasing concern regarding engineered nanoparticles' (ENPs) environmental impact is derived from their increasing production, unconventional properties and mostly unknown behaviour in the environment. As of now, environmental concentrations of ENPs cannot be measured by in-situ quantification due to analytical challenges. Consequently, the design of meaningful toxicity tests and ENP risk assessment have to rely on rational estimations of ENP environmental concentrations. In this study we present the design and evaluation of an ENP fate model capable of incorporating environmental complexity to predict realistic environmental concentrations of ENPs. This model is based on a designated fate model for ENPs in surface waters first developed by Praetorius et al. in 2012. As in the original model, heteroaggregation between the ENPs and suspended particulate matter (SPM) is assumed to be a key process determining ENP transport. Realistic conditions were introduced by defining variable SPM composition and concentration along the river and time dependent emissions. We evaluated our model by investigating the predicted fate of nano-TiO_2 in the lower Rhône River (France) since this river is characterized by a unique SPM variability. In order to constrain our model with a realistic SPM composition, the SPM parameters employed were derived from a recent sampling campaign of that river. Since the attachment efficiency - α_{hetero}, a fundamental parameter governing the heteroaggregation process, is not easily measured, we evaluated our model's predictions for all possible α_{hetero} values between the measured SPM and nano-TiO_2. Our model results provide spatially resolved concentration values for the free nano-TiO_2 particles as well as the nano-TiO_2 bound to SPM, both in the water and sediment compartments along the course of the river. The combination of all results was then analysed to obtain the most probable transport profile of nano-TiO_2 in the lower Rhône This novel approach provides a powerful tool for predicting realistic ENP concentrations in surface waters, thereby enabling the design of rational toxicity tests and supporting the risk assessment process of ENPs.

MO151

SimpleBox4nano predicts environmental behavior of nanomaterials

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A new version of the multimedia mass balance model SimpleBox has been released. In SimpleBox4 (platform presentation "SimpleBox Solution"), new insights in substance-specific transport and transformation mechanisms have been incorporated to allow exposure modeling of chemicals with a more complex structure than the traditional chemicalsubstances. Recently published approaches to model typical colloidal processes have been added to enable environmental fate simulation of nanomaterials. Functionality of the new "SimpleBox4nano" has been explored by modeling a suite of hypothetical nanomaterials, using slowly emerging measurement data from literature, as obtained from the Nanomaterial Registry (https://www.nanomaterialregistry.org). It appears that there are two dominant influences on exposure concentrations of nanomaterials in air, water and soil, which have not been accounted for in traditional multimedia mass balance models (i) rate of dissolution in water, and (ii) rate of aggregation and subsequent deposition. Particularly the latter may be important, since colloidal dispersions of nanoparticles in air and water are usually rather unstable and rapidly form homo- and heteroaggregates, followed by often rapid deposition from atmosphere and water column. The results of this study demonstrate the importance of quantitative knowledge of the (in)stability of colloidal dispersions under environmental conditions. Ideally, rate constants for transport by aggregation/deposition (e.g. from water to sediment) should be written as explicit functions of physical-chemical characteristics of the nanomaterials and water chemistry, using the well-established DLVO-theory of colloid stability. Practically, application of this theory to nanoparticles in environmental systems is still underdeveloped, so that predictions of environmental exposure concentrations need to be based on reported empirical observations.

MO152

Toxicity of fresh and aged suspensions of silver nanoparticles to Pseudokirchneriella subcapitata (Korshikov)

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Silver nanoparticles (AgNPs), with their demonstrated antimicrobial effects, are increasingly found in consumer products. However, incomplete information is known about their environmental impacts. Due to their multiple uses, especially in textiles, AgNPs are expected to enter the aquatic environment mainly through the wastewater collection and treatment system. In this study, the short-term chronic toxicity (72 h) to *Pseudokirchneriella subcapitata* (Korshikov) of eight commercial AgNPs (20 – 100 nm) was investigated in reconstructed freshwater according to ISO standard 8692 using a fluorimetric method and accounting for quenching phenomena. The relative importance of coatings and dispersants was assessed in addition to the effects of ionic silver. The experimental design focused on how aged AgNPs suspensions of 1, 4, 7, 14 and 21 days may behave differently from their pristine counterparts and thus influence their ecotoxicity depending on their changed chemistry through the ageing process. Recognizing transformation of NPs occurred during the aging process, solution and particle characterization complemented the determination of EC50 values of the exposed algal populations for each experiment. Total Ag was quantified by ICP-MS after acid digestion of the NPs suspensions, while the fraction of ionic and/or small Ag complexes/aggregates was measured by ICP-MS after filtration. The ionic fraction was determined via Ag ion-selective electrode. After analysis of variance, toxicity effects were deemed as averagely independent ($p < 0.05$) between treatments from the age of the suspension. Regardless, within a single treatment the age of the testing media influenced the ecotoxicological effects, which tended to increase at later time points thus with aging of the suspensions. In some cases, the AgNPs toxicity was equal to that generated by the Ag ionic form representing the reference positive control (6 ±

2 µg/L, n = 5). Particularly, the toxicity varied with the age of the suspension for uncoated NPs, but much less for coated NPs. As a general statement, it was observed that the ageing time of AgNPs suspensions may influence the resulting toxicity effects in a way that depends upon the characteristics of the single AgNP formulation.

MO154

Aqua-Nano project: Fate of engineered nanoparticles and effects on marine pelagic ecosystem

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Aqua-Nano project has been funded by the General Secretariat of Research and Technology, Hellenic Ministry of Education, Lifelong learning and Religious Affairs. This project will address the urged need to study the physicochemical and biological behavior of engineered nanoparticles in marine ecosystem. Engineered nanoparticles are materials which are produced purposefully by human activity and are less than 100 nm in size. Very little is, however, known about the fate, transport, and transformation of nanoparticles in aqueous systems. How and to what extent nanoparticles influence aquatic ecosystems is not yet clear. Aim of this project will be the development and applied an ecosystem approach for understanding the mechanisms of natural and engineered nanomaterial impacts on marine planktonic ecosystem. The core idea of the project is to use HCMR mesocosm facilities (http://mesoaqua.eu/cretacosmos) and a food web approach in order to study the effects of engineered nanomaterial on planktoning system. The use of mesocosm experiments is an innovative approach on ecosystem response and it will be the first time such approach will be used.. These in situ mesocosms represent a community level approach and will enable us to examine the bioavailability of specific nanoparticles on marine food web and also perform toxicity tests. Novel analytical chemical procedures and biological (eg. real time PCR) will be developed. Investigating the Behavior of Metal-containing Nanoparticles (NP) in Seawater Environments will be performed using Single-Particle Inductively Coupled Plasma Mass Spectrometry. The project will analyse the effects of silver and titanium nanoparticles on natural plankton communities. NP size distribution, number concentration, and metal mass fraction, for the NPs occurring in both the seawater and in marine biota will be analysed. Furthermore, the synergistic effect of co-occurrence on Silver nanoparticles and organic pollutants on marine pelagic ecosystem will be investigated.

MO155

A biodynamic description of the uptake and depuration of different sized CuO nanoparticles in the freshwater gastropod Potamopyrgus antipodarum

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Copper (Cu) and copper oxide nanoparticles (CuO NPs) are used in a wide range of industrial and consumer products; e.g. antimicrobial textiles, bioactive coatings and skin products. Consumers and ecosystems are therefore likely to be exposed to these particles, and previous studies have shown, that NPs from various sources will be released into the environment. The particles will eventually reside within sediment ecosystems where they can be inadvertently ingested by sediment-dwelling organisms. However, the environmental impact and dietary bioavailability of these particles remains largely unknown. **'In-house' synthesized CuO NPs (20nm) and commercial poly-dispersed CuO NPs (≈100 nm) was used to examine the accumulation kinetics of the freshwater gastropod, *Potamopyrgus antipodarum*, exposed to dietary copper oxide NPs or aqueous Cu (administered as $CuCl_2 \cdot H_2O$). Due to the small size and high surface-to-volume ratio of NPs, it is believed that the uptake of these particles will happen more easily than for the bulk material, with smaller size leading to a higher degree of uptake. Hence, the 20nm CuO NPs are expected to be more bioavailable for the organisms compared to the poly-dispersed CuO 100nm NPs.\n**20nm NPs was synthesized by reducing $CuCl_2 \cdot H_2O$ (aq) with ascorbic acid, followed by continuously stirring and heating for 16h. Polyvinylpyrrolidone (PVP) was added in order to stabilize the NPs, and they were characterized using several methods, including Transmission Electron Microscopy (TEM). The size distribution was 19.6±5.3nm and visual imaging showed that the particles were spherical, coated copper NPs. \nTo quantify dietary uptake, *P.antipodarum* was exposed for 2-4h to carbon-cleaned natural sediment, mixed with algae (*Nitzschia palea* (Kützing) W.Smith) to ensure a sufficient organic content, and subsequently spiked with a range of NP and Cu^{2+} concentrations. Elimination was determined over a depuration period of 15days. \nExperimental determination of unidirectional uptake and elimination rate constants using the biodynamic model permits modeling steady state Cu concentrations within the organism, which in turn can inform upon the potential toxicity of NPs. We will discuss how bioavailability is affected by the size of NPs using the biodynamic parameters.

MO156

Bioaccumulation of Cu2O NPs in Potamopyrgus antipodarum exposed to spiked biofilm

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Nanoparticles (NPs) possess new technological properties due to their increased reactivity and higher surface-to-volume ratio compared to their bulk counterparts. Engineered NPs come with different coatings which may affect the stability of the particles and their environmental fate. NPs are thought to sediment when introduced to natural aquatic environment, and subsequent uptake of the particles through digestion can therefore become a risk for benthic deposit-feeders. NPs are assumed to bind to organic compounds in the environment and thus to be incorporated into biofilms. Must surfaces in aquatic environment will be sporadically covered with biofilm. The use of spiked biofilm as a food source represents a realistic uptake route for benthic deposit-feeders. The aim of this study was to examine bioaccumulation and toxicity of Cu from biofilm spiked with Cu_2O NPs in the gastropod *Potamopyrgus antipodarum*. Biofilms were grown for 96 hours prior to spiking under controlled temperature and light conditions in natural river water and were then exposed to three different Cu forms (0.1 mg Cu/L) for 24 hours: non-coated and Polyvinylpyrrolidon (PVP) coated Cu_2O NPs, aqueous Cu ($Cu_{(aq)}$) was used as a reference. The size of the synthesized NPs was 8.93±6.9 and 10.68±7.9 nm for non-coated and coated Cu_2O NPs in artificial media, respectively. Bioaccumulation and toxicity (i.e., feeding rate) was tested on *P. antipodarum* exposed to contaminated biofilm for 72 hours. A biofilm concentration of 153.4 and 150.14 μg Cu/g DW biofilm for non-coated and coated NPs, respectively, at the end of the test, was achieved. The feeding rate of *P. antipodarum* was not significantly affected by exposures to any of the Cu treatments. None of the Cu treatments affected the bioaccumulation (body burden) significantly. NPs were also not accumulated significantly compared to $Cu_{(aq)}$. Results suggest that - since NPs (coated or non-coated) are not accumulated to a higher degree than $Cu_{(aq)}$ - released CuNPs will contribute solely to an overall increase in Cu concentration in receiving environments.

MO157

Nanoparticle post-exposure characterization in animal tissues: Enzymes lead the way

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The production and application of engineered nanomaterials (ENMs) is still increasing and the range of nano-composit consumer products has also multiplied, leading to increased potential for their environmental release. To appropriately characterize the fate, effects and ultimately risks of ENMs in environmental systems, methods are essential to characterize nanoparticles (NPs) in complex biological matrices. Most methods currently used are destructive, with acid destruction for instance additionally dissolving metal NPs. These conventional methods are thus limited in providing information about the effects of size, surface charge, coating, etc. on internal dose levels and fate of ENMs post-exposure. Here we used a combination of enzymatic tissue processing (ETP) followed by ICP-MS, TEM and AF4 to quantify and better characterize AgNP in tissues of the red earthworm *Lumbricus rubellus* after 28 day exposure to nominal concentrations of AgNPs (1.5, 15 and 150) or $AgNO_3$ (1.5 and 15) mg Ag/kg soil. For this, we compared total Ag burdens in ETP-extracted earthworm tissues with Ag levels obtained upon conventional microwave-assisted acid digestion method using routine ICP-MS. Results indicated concentration-dependent total Ag burden of 0.645 ± 0.141, 8.621 ± 0.138 and 15.480 ± 7.61 μg Ag/g (AgNP exposure) and 0.970 ± 0.435 and 8.315 ± 2.470 μg Ag/g ($AgNO_3$ exposure). Total silver in earthworm tissues processed enzymatically revealed comparable concentration-dependent body burdens as those processed via direct microwave-assisted acid digestion, amounting to 1.986 ± 1.822, 3.928 ± 2.275 and 6.641 ± 4.945 μg Ag/g (AgNP exposure) and 2.054 ± 2.132 and 6.968 ± 6.331 μg Ag/g ($AgNO_3$ exposure). TEM images of ETP extracted particles of AgNP exposed earthworms indicated the presence of electron-dense spheres consistent with images made of the AgNPs as-supplied. Further post exposure characterization of ETP-extracted particles using AF4 is underway and will be presented. The present study provides possibilities for improved post-exposure characterization of ENMs in tissues of exposed soil invertebrates. This will enhance the effectiveness of current risk assessment strategies.

MO158

Impact of cerium dioxide nanoparticles on a reconstructed aquatic ecosystem: a mesocosm study

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The growing interest in cerium dioxide nanoparticles (CeO_2 NPs) for industrial purposes (catalysis, coatings, solar cells, cosmetics…) has led to important volumes of production in the last few years. Environmental release is then expected, but NPs toxicity remains not well known. Ecotoxicological studies on CeO_2 NPs are emerging but are still too few to provide an overview on these particles environmental impact. Furthermore, the complexity of environmental conditions is not taken into account in standard studies, leading to a poor representativeness of NPs toxicity in natural conditions. The aim of the present study is to assess the toxicity of CeO_2 NPs on a simplified aquatic ecosystem using low NPs concentrations, as expected in natural aquatic environments, to provide a better understanding of their toxicity mechanisms. For this purpose, mesocosms comprising a complex primary compartment (bacteria, fungi and algae), and primary (dipteran larvae *Chironomus riparius*) and secondary (amphibian larvae *Pleurodeles waltl.*) consumers were used. This system allows the establishment of trophic relations and simultaneous exposure of the different species, coupled with continuous monitoring of physico-chemical parameters. Mesocosms' contamination consisted in a regular introduction of CeO_2 NPs for four weeks, to get to the final concentration of 1 mg/L of NPs. Toxicity on primary compartment was assessed as bacterial community diversity. Surviving chironomid larvae were counted at the end of the experiment, and growth inhibition was assessed as chronic toxicity endpoint. Mortality, growth inhibition and genotoxicity were assessed on amphibian larvae. No toxicity was observed either on primary compartment or on chironomids, but amphibian larvae were importantly impacted. Indeed, significant genotoxicity, growth inhibition and mortality were observed. Complementary experiments were also realized in order to determine possible mechanisms of toxicity on amphibian larvae. This work has been carried out within the ANR- P2N MESONNET project.

MO159

Effect of silver nanomaterials on microbial diversity in sewage sludge

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The rising use of silver nanomaterials (AgNM) increases the potential for environmental contamination. AgNM will inevitably reach the wastewater treatment plant (WWTP) were the AgNM adsorb mainly to the sludge. Currently, there is little information available about the effect of AgNM on the microorganisms in a WWTP. This study aimed to evaluate the effects of a spherical AgNM (NM-300K) and a nanorod (NM-302), both from the OECD Sponsorship Program, on the microbial diversity of sewage sludge of a simulated WWTP. A WWTP was simulated (OECD TG 303A) with a denitrification, nitrification and a settling tank. Over 10 days continuously NM-302 at 0.04, 1 and 4 mg/L and a NM-300K at 1 mg/L were dosed into the WWTP. The dissolved organic carbon (DOC) was measured daily in the influent and effluent of the different treatments. The influence of AgNM on the sludge microorganisms was investigated by observing the DOC elimination comparing the different treatments with the control. Additionally the microbial diversity was studied with two fingerprinting methods: polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and automated ribosomal intergenic spacer analysis PCR (ARISA-PCR). Eubacterial primers, for each method, were used to amplify the extracted DNA from sludge. Over the entire experiment no inhibition of DOC elimination by AgNM was detected. Even with continuous addition of 4 mg/L NM-302 for 10 days, the DOC elimination by the microorganisms was not inhibited. The DGGE pattern did not show differences in the bacterial ecological distribution within the different treatments, showing the same predominant groups of bacteria. However when using NM-302 at a concentration of 4 mg/L the intensity of the bands of some predominant bacteria decreased considerably, suggesting that the total population decreased in number. With ARISA-PCR, similar profiling was observed, however some groups of bacteria disappear at higher concentrations of the nanorod NM-302 and with NM-300K, while other groups seems to be favored. The study shows, that the conventional endpoint DOC elimination (OECD TG 303A) gave no evidence of an effect of AgNM on the microorganisms in sludge. A deeper analysis of microbial diversity gave first indications of a possible shift of individual microorganism populations. Further studies, to evaluate in deep the differences observed, must be conducted. The study was financially supported by the EU MARINA Framework 7 project.

MO160

Short-term effects of silver nanoparticles on periphytic communities

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Periphyton is a mixed microbial community of auto- and heterotrophic organisms embedded in an extracellular polymer matrix. It is involved in ecosystem functions such as primary production and nutrient cycling. Although periphyton has been identified as a probable sink of nanomaterials in aquatic environments, its sensitivity to nanomaterials is unknown. In this study, we assessed the short-term effects of citrate-coated silver nanoparticles (AgNP, 35 nm diameter) and silver ions (Ag^+, dosed as $AgNO_3$) on periphyton through their effects on photosynthesis, respiration, and potential activity of three extracellular enzymes: β-glucosidase (β-GLU), leucine aminopeptidase (LAP) and alkaline phosphatase (AP); which are involved in nutrient acquisition. Glass slides colonized with natural periphyton were placed in microcosms filled with reconstituted freshwater (pH 7.5) and exposed to concentrations ranging from 12.5 to 200 μM AgNP or from 1.25 to 25 μM $AgNO_3$, during 2 hours in the dark at 15 °C. After exposure, the periphytic community was scraped off the slides, suspended, and used in short-term bioassays. Photosynthetic activity was assessed by fluorometry, CO_2 produced during respiration was measured colorimetrically, and potential enzyme activities were assayed with fluorescent substrate analogues. The silver ion ligand sodium 2,3-dimercaptopropanesulfonate (DMPS, 25 µM) was used to assess the contribution of Ag^+ in causing toxic effects. Based on total silver mass, $AgNO_3$ was more toxic than AgNP for both endpoints. The AgNP concentration causing a 50% inhibition (EC_{50}) was 83 μM for photosynthesis and 22 μM for respiration. In the case of $AgNO_3$, EC_{50} values were 3 μM for photosynthesis and 4 μM for respiration. Potential enzymatic activities were also inhibited by both AgNP and $AgNO_3$. β-GLU and LAP activities decreased with increasing concentration of silver. EC_{50} values were 17 μM AgNP and 2 μM $AgNO_3$ for β-GLU, and 23 μM AgNP and 2 μM $AgNO_3$ for LAP. AP activity did not follow a concentration-dependent decrease as the two other enzymes. Based on these values, the toxicity observed could not be solely attributed to the measured fraction of free Ag^+ in the AgNP suspensions (1.5 – 3 %). Experiments with DMPS prevented AgNP toxicity to most endpoints, indicating that the toxic effects were mainly caused by Ag^+. The exception was LAP, which was directly affected by AgNP.

MO161

Ecotoxicity of a novel anti-corrosion engineered nanomaterial in two invasive bivalve species

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Nowadays, corrosion is one of the most relevant problems affecting underwater metallic structures (e.g. ship hulls, oil platforms). To minimize these problems the metallic substrates have been protected with specialized anti-corrosion coatings which contain active compounds, such as corrosion inhibitors. The corrosion inhibitor mercaptobenzothiazole (MBT) is amongst the most widely used in paints, showing high toxicity for organisms and humans. Recently, important insights were given in terms of encapsulation of active compounds, as a result of the banishment or restriction of environmentally harmful compounds (e.g. TBT). The encapsulation is a process used to prevent the interaction of active compounds with paint formulations, to promote their controlled release and to provide protection against the environment. This process may involve "smart" and novel engineered nanomaterials, such as the layered double hydroxides (LDH), also called anionic clays, in which active compounds are encapsulated until the conditions are adequate to be released (e.g. coating degradation, pH changes). When released, active compounds and nanocontainers come available to organisms, but little is known about their fate and toxicity for soft-bottom benthic communities, especially bivalves, despite the eco-friendly labeling of nanocontainers. In this study, two invasive species were selected, one from the marine environment (edible Manila clam *Venerupis philippinarum*) and another from the freshwater environment (Asian clam *Corbicula fluminea*). To assess the potential effects of the anti-corrosion LDHs and the free active compound (MBT), 96h exposures were carried out in both bivalve species. For each species, mortality, clearance rate, survival on air, condition index and biomarkers (LPO, CAT, GST, CBE, ChE and total protein) were assessed.

MO162

Cytotoxicity and immune response of mussel mytilus galloprovincialis after exposure to CdTe QDs and Cd2+

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Quantum Dots (QDs) are nanocrystals (2 to 9.5 nm in diameter) composed of a semiconductor core and shell that can be modified by the addition of different functional groups. Due to their recognized specificity on cellular targets, strong

fluorescence and resistance to photobleaching, they are used in electronic, nanomedicine, pharmacy and biology. However, these nanoparticles (NPs) can be released into the marine environment, where their toxicological effects and mechanisms of action are unclear. In particular, filter-feeding organisms are target groups for NPs toxicology and the marine bivalve *Mytilus* spp. represent an important model for investigating the toxic effects of NPs in marine invertebrates. Accordingly, the main objective of this study was to analyse the immune response of *Mytilus galloprovincialis* exposed to 10 μg.L^{-1} of CdTe QDs in comparison to their ionic counterpart for 3, 7 and 15 days. Several cytotoxic and genotoxic parameters were analysed, namely density and viability of circulating hemocytes, lysosomal membrane stability (LMS), differential hemocyte counts, micronucleus frequency, frequency of nuclear anomalies and DNA damage (comet assay). This study showed that the accumulation of QDs induces cellular stress and modulates the immune response of mussels *M. galloprovincialis*. Hemocytes lysosomal integrity and cell viability decreased after 3 days of exposure in mussels exposed to both Cd forms, remaining unaltered until the end of exposure. In terms of LMS and density, of significant differences were observed between QDs and Cd^{2+} exposures. On the other hand, the results on differential hemocyte counts and frequency of nuclear anomalies showed different responses on hemocyte types, dependent on the Cd form. After the 15 days of exposure, QDs induced a significant decrease in the percentage of circulating eosinophils and an increase in basophils. In contrast, Cd^{2+} caused a decrease in the proportion of both cell types and increased circulating hyalinocytes. These results indicate that the mechanism of immunotoxicity of QDs may be due to Cd^{2+} dissolution, even though the responses of the types of hemocytes vary according to the form of Cd. Overall, this study showed that *M. galloprovincialis* hemocytes are a suitable model for investigating the effects of NPs in marine invertebrates.

MO163

Multibiomarker assessment of the long term effects of dissolved and nanoparticulate silver (nAg) on zebra mussels: influence of feeding

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Incorporation of silver nanoparticles (nAg) in everyday products is expanding quickly, and concerns on their ecological consequences are growing. nAg PEC in surface waters are currently expected to be below the μg/L range. If some studies showed nAg toxicity, even in the low μg/L range on several organisms (including bivalves), solubity issues are forcing us to compare nAg toxicity to dissolved Ag toxicity to try to distinguish specific nAg effects. Feeding is another scarcely studied parameter that could influence Ag toxicity by changing its bioavailability to organisms. The aims of our study were 1) to test nAg effects at low concentrations, 2) to observe an hypothetic modulation of toxicity associated with feeding, and 3) to compare nAg and dissolved Ag toxicity mechanisms. For that, nAg (60 nm, citrate coated) and dissolved silver ($AgNO_3$) effects were assessed on zebra mussels (*Dreissena polymorpha*) following a 14-day exposure to 0.5 and 5 μg/L, with and without lyophilized alga feeding. Mussels were sampled after 7 and 14 days and the haemocyte immunological functions and a wide range of biomarkers in digestive glands were measured (antioxidant and detoxification systems, cellular damages, energetic reserves). Results were synthesized using the Integrated Biomarker Response (IBR) tool. The first results showed a significant toxicity of both dissolved and nanoparticulate Ag on haemocyte immunological parameters. For both sampling times, stress levels as revealed by IBR calculation were consistently higher for unfed mussels. A dose-dependent decrease of cell viability, phagocytosis capacities and ROS production, more pronounced for nAg than for $AgNO_3$, was observed, denoting a potential increase in mussel sensibility to pathogens. On the contrary, the activation of the immune system for fed mussels happened only at day 7, but the stress levels went back to normal after 14 days, resulting from a lowered bioavailability of Ag or a better mussel fitness. As a whole, this work currently suggest that 1) long-term exposure to low concentrations of Ag could inhibit durably mussel immune defenses, 2) feeding decreases Ag toxicity, and 3) at equivalent nominal concentrations, nAg seems to be more toxic than dissolved Ag, suggesting differences in penetration, accumulation and action mechanisms of the two forms in organisms. Those results will be completed by water and internal concentrations of Ag and biomarker measurements into digestive gland.

MO164

Remote effects in charophyte cell of Nitellopsis obtusa after short term exposure to CuO nanosuspensions

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Survival of the cells of macrophytic green algae *Nitellopsis obtusa* exposed to 100 mg/L CuO nanosuspension (< 50nm, Sigma-Aldrich) for at least 5 minutes decreased substantially after 8 days, while the rewash from noticeably toxic concentrations of Cu^{2+} (as $CuSO_4$), after short exposure (up to 6 hours), prevented cell mortality [Manusadzianas et al., 2012]. To explore whether toxicological threshold of exposure to nCuO does exist, the charophyte cells (n=60/treatment) were exposed for periods as short as 5 s to 24 h at sonicated 30 mg/L nCuO, then rewashed in media up to 72 days. Two controls, one with media, another with 30 mg/L nCuO (permanent exposure) were used. For comparison of quantal (mortality probabilities) effects of two single exposure durations the exact Fisher test were used (

MO165

A droplet based microfluidic device for single paramecia cell toxicity testing - a case study with silver nanoparticles

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In microfluidic circuits droplets of analytes which are embedded in an emulsion liquid are infused into fine tubes. This represents a promising technique for non-invasive analysis of single cells or populations. It enables fast analysis of high replicate numbers while operating in very low volumes, down to picolitre range. Here we present a simple method to encapsulate single *Paramecia* cells in a total volume of about 100 nL, supplied with silver nanoparticles and a viability dye, which is transformed to a fluorescent compound by living cells. The aim is to observe the viability of the cells after exposure by measuring the fluorescence of the viability dye in-situ using optical techniques as a toxicological endpoint. Different cell densities and injection rates were tested in order to maximize the efficiency of introducing single cells into droplets, which is challenging because of the mobility of the cells and hence a biased distribution of cells in the liquid. Spectra of every single droplet were recorded and drops were computationally counted and labeled. The number of encapsulated cells was determined based on metabolic activity by this method and results were in accordance with counterchecks with a stereomicroscope. As a result we were able to record cell division of one confined paramecia cell. Microfluidic circuits are predestinated to be used for high sample capacity and for mixture toxicity.

MO166

Transformation and distribution processes governing the fate and behaviour of nanomaterials in the environment: an overview

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To develop a more complete understanding of the potential risks posed by manufactured nanomaterials, it is necessary to further examine their transport and fate in all environmental compartments. At present analytical chemistry methods are limited when it comes to detecting low concentrations of nanomaterials. Furthermore, distinguishing between naturally occurring, anthropogenic and manufactured nanomaterials in an environmental matrix poses additional analytical-chemical challenges. This situation leaves a gap between the present scientific state-of-the-art and the increasing demand for reliable measured or predicted environmental exposure concentrations for environmental risk assessment of ENMs. To fill this gap within a limited time frame modeling appears to be a suitable approach. Chemical fate modeling for nanomaterials is still in its infancy. Informed choices on the basis of available knowledge are needed during model formulation and development to ensure the reliability of predicted environmental concentrations of nanomaterials. A major knowledge gap hampering the further development of such model-based approaches is our current lack of understanding of the interplay between the novel physico-chemical properties, exhibited by many nanomaterials, the properties of the surrounding media/matrix and the underlying processes that determine particle behaviour. Here we identify and summarize key processes governing the fate and behaviour of nanomaterials in the environment. This is done through a critical review of the present state-of-knowledge. We describe the (photo)chemical, physical or biologically mediated transformation of manufactured nanomaterials from their pristine form (as produced or in a product) due to degradation, aggregation, agglomeration, or through association with dissolved, colloidal or particulate matter present in the environment. Specific nanomaterials are used as case studies to illustrate these processes where relevant. Key environmental processes are identified and ranked and key knowledge gaps are identified, feeding into the longer-term goal of improving the existing models for predicted environmental concentrations.

MO167

Number concentration and size distribution of nanoparticle from suspension by transmission electron microscopy

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Transmission electron microscope (TEM) is the nanoanalytics toolbox and can provide accurate information on nanoparticle (NP) number size distribution, number particle concentration at low concentrations (*ca.* ppt to ppb range) and small sizes (*ca.*< 20 nm) as well as chemical composition. However, the high capabilities of TEM are limited by the traditional sample preparation based on drying a small volume of suspension of NPs on a TEM grid. This method is limited by low recovery of NPs, formation of aggregates during the drying process, and thus, the complete misrepresentation of the NP suspensions under consideration. The present work presents a fully validated sampling technique for TEM that overcomes these shortcomings and allows full recovery and representativeness of the NPs under consideration by forcing the NPs into the TEM grid via ultracentrifugation and strongly attaches the NPs to the TEM grid by surface functionalization of the grid with a positively charged polymer (poly-l-lysine). The high efficiency of the analysis is demonstrated by the uniformity of the NP distribution on the TEM grid (that is low variability between the number of NPs counted on different images on different areas of the grid), the high recovery of the NPs up to 71%) and the good correlation (R>0.95) between the mass and number concentrations. The applicability of the protocol to detect and measure the number concentration of NPs in natural water was demonstrated on natural water spiked with AuNPs at different concentrations. AuNPs were uniformly distributed on the TEM grid (CV< 0.2) and good correlation between number and mass concentrations (R^2~ 0.9) and high recovery (75%-85%) were achieved. Therefore, for the first, we developed and applied a fully validated sampling protocol that enables the use of the full capabilities of microscopy tools to quantitatively and accurately determine the number size distribution and number concentration of NPs at environmentally relevant low concentrations (*i.e.* 0.34-100 ppb). This approach is of high environmental relevance and can be applied widely in environmental nanoscience and nanotoxicology for (i) detection of NPs in environmental systems at low concentrations, (ii) measuring the number concentration dose in nanotoxicological studies, and (iii) accurately measure the number size distribution of NPs, a key requirement for the implementation of the European Commission definition of nanomaterials.

MO168

Transformation and retention of silver nanoparticles through engineered inorganic wastewater treatment media

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Nanotechnology is an expanding industry with important implications for the global economy. Silver nanoparticles (AgNP) have assumed dominance within this market, with widespread use in industry, medicine and commercial applications. Projections indicate that at least one third of all AgNP containing products have the potential to leach silver into wastewater streams. As such, wastewater treatment plants (WWTP) play a key role in mitigating the impacts of AgNP upon receiving aquatic environments. A concern for WWTP is the potential for AgNP to affect the operation and efficiency of unit processes. Also, the capability of treatment processes to remove nanoparticles from wastewater streams is yet to be fully understood and quantified; this includes inorganic filtration techniques, which to date have not benefited from the same close research scrutiny as their biological counterparts. Using laboratory columns, operated under steady state flow conditions, this investigation aims to evaluate the fate and transport of AgNP through a variety of inorganic filtration substrates. Research will focus upon the physical and chemical interactions of AgNP within the experimental environments. Analysis of AgNP influents and filtered effluent samples has been conducted using transmission electron microscope, energy-dispersive X-ray spectroscopy and inductively coupled plasma optical emission spectrometry in order to characterise the nanoparticles. Preliminary results indicate that AgNP can exhibit varying, yet distinct degrees of mobility and persistence depending upon the filtration media employed. Also, AgNP appears to be less susceptible to ligand interaction than other forms of silver speciation exposed to the same environmental conditions. Column data suggests AgNP mobility in the columns to be closely associated to the surface charge of both the nanoparticles and of the media environment; as such the different capping agents commonly employed in the manufacture of AgNP, such as citrate and PVP, are likely to play an influential role in the mobility and persistence of AgNP through these filtration processes. This work aims to contribute data pertaining to the effects of inorganic treatment techniques and variability of system conditions upon AgNP behaviour and subsequent silver speciation and has the potential to assist future toxicological studies by providing speciation and concentration projections for AgNP at varying stages through a WWTP and into the environment.

MO169

Dispersion stability of CeO2 nanoparticles in different aqueous media and implications for freshwater algae ecotoxicity testing

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In the present study, an aqueous dispersion of CeO_2 nanoparticles (NPs) was evaluated for its stability in a range of freshwater ecotoxicity media, with and without the presence of natural organic matter (NOM). The potential ecotoxicity of the CeO_2 dispersion was then investigated using the freshwater algae *Pseudokirchneriella subcapitata*. The CeO_2 NPs (10 wt%) were supplied as an aqueous colloidal suspension (pH 8.5) and stabilized with excess anionic polymer stabilizer. Relevant physicochemical properties of the CeO_2 NPs were investigated before use in fate and effects studies. The crystallite size of the particles, determined using transmission electron microscopy, was 3-5 nm with aggregates up to 20 nm observed. Energy-dispersive X-ray spectroscopy indicated trace amounts of Au and Co impurities. HAADF-STEM in Z-contrast mode was used to image the PAA stabiliser present on the surface of the CeO_2 NPs. A Malvern Zetasizer indicated the stock CeO_2 NP dispersion had an average particle size of ~80 nm (dynamic light scattering; DLS) and a zeta potential of -25 mV, indicating the dispersion exhibits good colloidal stability. The stability (zeta potential) and aggregation (DLS) of the CeO_2 NP dispersion was investigated over time following dilution (0.01 and 1 mg/L) into a number of common freshwater ecotoxicity media; moderately hard reconstituted water (general media), TG201 (freshwater algae) and M7 (*Daphnia magna*). Influence of NOM from the Suwannee River (SR-NOM) on dispersion stability was also investigated for each media type. The ecotoxicity of the CeO_2 NPs to the freshwater microalga *Pseudokirchneriella subcapitata* was assessed using a modified version of the ISO algal growth inhibition method. In order to overcome 'shading' of the algal cells when measuring algal growth by fluorescence, the chlorophyll pigments were extracted into ethanol and filtered prior to analysis of absorbance by UV-vis spectroscopy. In order to account for any toxic contribution from the anionic polymer stabilizer present in the stock solution an NMR-based quantification method was established using pure polymer stabilizer. Linear calibration curves (R^2 0.999 from 0.01-100 mg/L) were established and used to determine the free polymer stabilizer concentration in the CeO_2 stock solution. In addition, the concentrations of particulate CeO_2 and dissolved Ce ions were determined by ultracentrifuging diluted aliquots of the stock solution and analysing the supernatant by ICP-MS.

MO170

UV-mediated transformation of C60 fullerene in top soil

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Owing to their innovative properties, fullerenes are considered as some of the most promising materials in emerging nanotechnology and they are currently applied in several fields such as electronics and household applications as well as cosmetics and organic photovoltaics. The expected increase in production is likely to cause increasing release of such materials into the environment and fullerenes have been suggested to enter the terrestrial ecosystem by direct release (e.g. leaching from fullerenes-containing materials) as well as deposition from the atmosphere and transport through aqueous media. Fullerenes are therefore expected to accumulate in the top soils but their environmental fate and behavior are still largely unknown. Sunlight is likely to play a major role in the transformation of fullerenes and UV-mediated transformation of C60 has already been reported in organic solvents and in aqueous solution. Data are missing, however, concerning the possible degradation of these compounds in soil environment which is expected to act as a sink for the fullerenes in the environment Therefore the aim of the present study was to investigate the degradation of C60 fullerenes in the top soil environment in presence of UV light. Sandy soils differing in texture and organic carbon content were amended with C60 at the concentration of 200 ppb and irradiated with UV light for several weeks. The samples were then extracted at specific time interval with both polar and non-polar solvents and analyzed with HPLC-UV-MS. Non-irradiated soil samples were incubated as controls and C70 fullerene was used as an internal standard for the assessment of the degradation rate. In addition, high-resolution mass spectrometric analysis was applied for the determination of both polar and non-polar transformation products.

MO171

Importance of particle dispersion and ionic environmenton on silver nanoparticle toxicity in the embryonic zebrafish

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The mechanism of action of silver nanoparticles (AgNPs) is unclear due to the particles' strong tendency to agglomerate. Preventing agglomeration could offer precise control of the physicochemical properties that drive biological response to AgNPs. In an attempt to control agglomeration, we exposed zebrafish embryos to AgNPs of 20 or 110 nm core size, and polypyrrolidone (PVP) or citrate surface coatings in media of varying ionic strength. AgNPs remained unagglomerated in

62.5 µM CaCl2 (CaCl2) and ultrapure water (UP), but not in standard zebrafish embryo medium (EM). Zebrafish embryos developed normally in the low ionic strength environments of CaCl2 and UP. Exposure of embryos to AgNPs suspended in UP and CaCl2 resulted in higher toxicity than suspensions in EM. 20 nm AgNPs were more toxic than 110 nm AgNPs, and the PVP coating was more toxic than the citrate coating at the same particle core size. The silver tissue burden correlated well with observed toxicity but only for those exposures where the AgNPs remained unagglomerated. Our results demonstrate that size- and surface coating-dependent toxicity is a result of AgNPs remaining unagglomerated, and thus a critical-design consideration for experiments to offer meaningful evaluations of AgNP toxicity

MO172

Particle characteristic dependent effects of titanium dioxide nanoparticles on Daphnia magna and Gammarus fossarum

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Peer-reviewed studies display a substantial variability in the ecotoxicity of titanium dioxide nanoparticles ($nTiO_2$)

MO173

Assessing the toxicity of silver nanoparticles in lentic (static) and lotic (flowing) freshwater environments

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Given the widespread use of silver nanoparticles (Ag NP), their release, whether accidental or intentional, into the environment is inevitable. At this point, Ag NPs threat to freshwater ecosystems is unclear. Current research shows NP toxicity is affected by water chemistry, the presence of organic matter, sediments and other possible influences, such as hydraulics. As such, there is a need to further the understanding of NPs' fate and toxicity in the aquatic environment. This research investigates conditions that affect Ag NP toxicity in lentic & lotic freshwater environments, in an attempt to identify what environmental scenarios may lead to higher hazard, using the Californian blackworm, *Lumbriculus variegatus* as a model organism. Survival, locomotive behaviour, and NP physicochemical characteristics are being assessed of Ag NP (0-30 mg l^{-1};polyvinylpyrrolidone (PVP) coated; 0.02 %wt, nominal size 30-50nm) and silver nitrate ($AgNO_3$;0-240 $\mu g\ l^{-1}$) in both static and turbulent 'environmentally relevant' freshwaters of different chemistries. Results indicate both Ag NP and $AgNO_3$ were acutely toxic to *L. variegatus* in static OECD 225 test water, though the latter was an order of magnitude more toxic (96hr LC50 values of 4.1 mg l^{-1} and 28.2 $\mu g\ l^{-1}$, respectively). There was evidence of locomotive impairment in *L. variegatus*, in static sub-lethal concentrations of Ag NP (3-15 mg l^{-1}) and $AgNO_3$ (20-120 $\mu g\ l^{-1}$), i.e. a 20-60% reduction in successful body reversal (96hrs). Nanoparticle tracking analysis (NTA) of agglomeration/aggregation size (A/A) of Ag NP (5mg l^{-1}) in static OECD 225 medium indicated a median A/A size of 157:116;208nm (median:25;75 percentiles;time=0hrs). The A/A size significantly decreased in low ionic strength (IS) 5%OECD225 medium 68:39;82nm (Man Whitney, P< 0.05). 24hr trials of turbulent flow systems, investigating method suitability, indicated no detrimental impact to *L. variegatus* survival or locomotive behaviour. Ongoing work will continue to investigate flow systems and how 'environmentally relevant' water chemistries, and NP sediment interactions affect the waterborne toxicity of Ag NPs. Results indicate Ag NP to be toxic to freshwater invertebrates in static conditions, however, realistic exposure conditions need to be considered in more detail when assessing environmental risk.

MO174

Response of Chironomus riparius to silver ions and silver nanoparticles

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Although nanoparticles (NPs) have always been present in the natural environment and were used throughout human history, it was only in modern times that there was a deep interest in their potential applications. Since it became technologically feasible to mass produce them, there has been an increasing and widespread use of NPs. Their continued use and ubiquity in every day products will surely lead to the increase of their release to the environment, reaching the aquatic medium, with sediments being the ultimate laying basin. Among NPs, silver nanoparticles (Ag-NPs) are one of the most used, notably due to their potential antibacterial properties. This study aims at evaluating the toxic effects of Ag-NPs and their respective ionic form (Ag^+) on the different life stages of the midge *Chironomus riparius* along its life cycle, and estimate how the exposure route affects the toxicity (spiked sediment and spiked water). To assess toxicity, acute, chronic and sub-chronic hatching tests (48 hours, 28 days and 6 days exposures, respectively) were performed, and the effects on survival, reproductive output, growth rate, emergence and hatchability were evaluated. Results showed that Ag^+ is more toxic than Ag-NPs and that the 1st instar larval stage is the most sensitive of the life stages. These assays proved that the exposure route is crucial in assessing the toxicity and bioavailabity of NPs and that the different life stages have different responses and sensitivities to contaminants.

MO175

Influence of nanoparticle shape on Cu bioaccumulation in the deposit feeder, Capitella teleta

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There have been few studies of the relationship between nanoparticle shape and bioaccumulation. In the present study, bioaccumulation dynamics was modeled in a deposit feeder, *Capitella teleta*, following exposures to sediment amended with nanosized copper oxide (spheres, rods or platelets) or dissolved Cu ions. After 7 days of exposure, Cu was accumulated in worms with weight-specific body burdens (μg Cu/g dry weight worm) of 13.3 ± 5.5 for controls, 65.5 ± 30.6 for dissolved ions, 57.8 ± 16.4 for spheres, 143.0 ± 43.2 for platelets and 235.4 ± 26.9 for rods. The results indicate that all nanosized CuO particles were bioavailable and bioaccumulated in *C. teleta*. Compared to dissolved ions, CuO rods were more readily accumulated in worms as assessed both from measured body burdens or calculated uptake rate constants. There were no statistically significant differences in bioaccumulation between dissolved Cu and the other CuO particles. However, Cu was accumulated faster and to a higher level for CuO rods than for CuO spheres. Based on Cu mass concentration CuO rods resulted in the highest Cu bioaccumulation in *C. teleta*, whereas CuO spheres had the highest bioaccumulation based on Cu particle number. We further suggest that in order to better understand the effects of nanoparticle shape on bioaccumulation in aquatic organisms, we need to develop easily implementable and affordable methods to characterize NPs in sediment to relate NPs body burden to dose.

MO176

Influence of particle shape and size on bioaccumulation and effects of Copper oxide nanoparticles in two sediment-dwelling worms

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The use of Copper oxide nanoparticles (CuO NPs) is increasing, thus causing concern about their environmental impacts. Here we examined the toxicity and bioaccumulation (in whole organisms and in five subcellular fractions) of CuO NPs of different size and shape in the sediment-dwelling worms *Nereis diversicolor* and *Lumbriculus variegatus*. In addition, CuO microparticles (CuO_{micro}) and aqueous Cu (Cu_{aq}) were used as references. Worms were exposed to sediment spiked with Cu_{aq}, CuO_{micro} or CuO NPs of different sizes and shapes ($CuO_{6nm\text{-}spheres}$, CuO_{100nm}, $CuO_{spindles}$ and CuO_{rods}) for 10 days (three experiments). In *L. variegatus,* bioaccumulation of either of the Cu forms tested (Cu_{aq} and $CuO_{6nm\text{-}spheres}$) was limited, whereas *N. diversicolor* more efficiently accumulated Cu from the sediment, dependent on Cu form added to the sediment (Cu_{aq}>CuO_{100nm}>CuO_{micro}). In contrast, CuO NP shape had limited effect on Cu bioaccumulation ($CuO_{6nm\text{-}spheres}$, CuO_{rods} and $CuO_{spindles}$) in *N diversicolor*. Neither Cu_{aq} nor CuO_{NP} affected *L. variegatus* (burrowing behaviour, survival). In contrast, Cu affected survival of *N. diversicolor* depended on Cu form (Cu_{aq}>CuO_{micro}, no effect of CuO_{100nm}). Weight-specific growth rate of *N. diversicolor* was affected by exposure to Cu_{aq} and $CuO_{spindles}$, but not by exposure to $CuO_{6nm\text{-}spheres}$ and CuO_{rods}. Distribution of Cu in subcellular fractions of *L. variegatus* differed slightly between the two Cu forms tested ($CuO_{6nm\text{-}spheres}$ and Cu_{aq}). Similarly, the subcellular distribution of Cu in *N. diversicolor* was dependent on Cu form, and distribution of Cu_{aq} was distinctly different from that of the particles tested (CuO_{100nm} and CuO_{micro}). Distribution of Cu_{Aq} differed between the two worms, such that Cu_{Aq} was mainly found in metallothionein-like proteins in *L. variagutus* and in metal rich granules in *N. diversiocolor*. Thus, representing different detoxification mechanisms in the worm species tested. Together these findings indicate that there is a difference in response between the two worm species and between Cu_{aq} and particulate CuO (both nano- and micrometer-sized). Generally, Cu_{aq} was found more bioavailable and toxic than CuO particles, especially for *N. diversicolor*. However, more studies on differences among species and effects of metal form, particle size and shape are needed. Only then can we ensure that we are able to assess the risk of NPs adequately and use nanotechnology wisely.

MO177

Factors triggering the combined toxicity of nanosized titanium dioxide and heavy metals

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As an ingredient in many consumer products (e.g. paints and sunscreens) titanium dioxide nanoparticles ($nTiO_2$) may enter aquatic ecosystems *via* wastewater treatment plant effluents, where they probably co-occure with other stressors, such as heavy metals. The adsorption of free ions onto $nTiO_2$ surfaces may reduce heavy metal toxicity in some cases, due to their removal from the water phase. However, agglomerates of $nTiO_2$ – together with the adsorbed metal ions – may actively be ingested by filter feeding organisms, such as *Daphnia magna*, enhancing the overall toxicity. In this context, the present study investigated shifts in copper (Cu) toxicity in presence versus absence of $nTiO_2$ after an interaction period (=aging) under different conditions. Thus, the influence of ionic strength and pH on the combined toxicity of $nTiO_2$ and Cu was assessed during 72-h acute toxicity test using *D. magna*. Briefly, the presence of $nTiO_2$ reduced Cu toxicity of the mixture aged in pure deionized water to an extent comparable to an unaged mixture of both stressors. The same was observed for an aging in test medium at pH 6. Contrary, 72-h aging in test medium at pH 8 lead to a 70% enhanced Cu toxicity following 48 h of exposure in presence compared to absence of $nTiO_2$. The supplemental analytics suggest, that the test organisms finally ingest numerous $nTiO_2$ agglomerates together with high amounts of absorbed Cu ions. Hence, nanonoparticle agglomerates may contribute meaningfully to the uptake and effects of other chemical stressors, while the environmental conditions during aging determin whether the toxicity of the mixture is increased or decreased by the $nTiO_2$.

MO178

A comparison of the acute and chronic toxicity of three silver nanoparticles and AgNO3 to Daphnia magna: implications for regulation and wide-level screening

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Silver nanoparticles (Ag NPs) are of growing interest due to their use in a wide range of applications. Currently Ag NP production accounts for over 30% of NP technology output. Questions have therefore been raised regarding environmental risks following Ag NP release. This study assessed how different Ag NP surface coatings and characteristics can affect toxicity. Polyhedral Ag NPs with mean core diameters of ~10 nm with three different coatings (ie. PVP, PEG and citrate) were used, in parallel with $AgNO_3$. The biological effects of the 3 Ag NPs and $AgNO_3$ were assessed using *Daphnia magna* in 48-hour static non-renewal OECD 202 immobility tests. LC_{50} were 0.9 μg/ L for $AgNO_3$ and between ~5-25 μgL^{-1} for the Ag NPs. 21-day *D. magna* reproduction studies followed OECD 211 protocol. Effects here were shown on moulting, reproduction and growth at concentrations from 0.3 μgL^{-1} for $AgNO_3$ and ~1-4 μgL^{-1} for the Ag NPs. Toxicity rank varied for each exposure type, acute and chronic exposure toxicity ranks were PVP-Ag NP > Cit-Ag NP > PEG-Ag NP and Cit-Ag NPs > PVP-Ag NPs > PEG-Ag NPs, respectively. Chronic sub-lethal effects between $AgNO_3$ and Ag NPs differed regardless of concentration as toxicities displayed for the different endpoints varied. Exposure to Ag NPs resulted in significatnly smaller *D. magna* length, before reducing offspring number; no effects were observed on total moults over 21 days, however $AgNO_3$ exposures showed a significant reduction in offspring before length and significant reduction in *D. magna* moults. Results obtained suggest potential different modes of action for acute and chronic effects, and for $AgNO_3$ and Ag NPs. Therefore extrapolation of acute data sets to chronic modelling or regulations may be ill-advised. The data also implies Ag^+ release from Ag NPs cannot fully explain their toxicity and using adapted Ag^+ toxicity models for Ag NP impact assessment may have inherent shortcomings. The authors acknowledge funding from NERC and contributions from other partners in this project.

MO179

Characterizing nanoparticle exposure and effects in the epibenthic crustacean, Hyalella azteca

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Manufacturednanoparticles (NPs) are expected to make their way into the aquatic environment where sedimentation of particles will likely occur, putting benthic organisms at particular risk. Therefore, organisms such as *Hyalella azteca*, an epibenthic crustacean which forages at the sediment surface, is likely to have a high potential exposure compared with water column organisms such as *Daphnia magna*. In general, *H. azteca* is more sensitive to NPs compared with *D. magna*; however, NP toxicity is particle specific. *H. azteca* is highly susceptible to Zinc Oxide (ZnO) NP exposure, with ZnO NPs demonstrating greater potency compared with the corresponding metal ion, Zn^{2+}. Silver (Ag) NPs act differently, with a lower potency compared to Ag ions. Our ongoing studies are comparing the toxicity of these particles to *H. azteca* in different sediment types and characterizing their transformation in these sediments. Preliminary results suggest that the presence of a substrate, including sand, decreases the toxicity of nanoparticles. These studies will help us better understand the susceptibility of benthic organisms to nanomaterials in realistic exposure scenarios. The views expressed in this presentation are those of the authors and do not represent those of U.S.EPA.

MO180

The effects of abiotic factors on the toxicity of nanoparticles to zebrafish (Danio rerio) larvae

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It is well established that water chemistry can influence the bioavailability and subsequent toxicity of contaminants. To enable the development of exposure models for nanoparticles (NPs) it is essential that the effects of these abiotic factors are established. Larval zebrafish (*D. rerio*) were used to assess the effects of water hardness (as Ca), pH, $NaCl^-$ and humic acid (HA) on the toxicity of TiO_2 NPs (anatase, 8 nm and P25 rutile/anatase, 21 nm), Ag NPs (15 nm) and multi-walled carbon nanotubes (MWCNT), supplied as part of the E.C. FP7 MARINA project, along with appropriate controls. Larvae were exposed in a semi-static design using bespoke exposure chambers that enabled dispersion of the NPs whilst ensuring animal health. Changes to water chemistry did not affect the toxicity of any TiO_2 material (survival generally >90 % throughout) with limited effect upon MWCNT (small decreases in survival with >Ca and HA). However, increased water hardness (150 mg Ca l^{-1}, added as $CaCl_2$) resulted in increased mortality (>80 %) which was not seen in $AgNO_3$ exposed larvae ($P < 0.05$). Subsequent experiments with $Ca(NO_3)_2$ confirmed that toxicity was related to Ca and not the Cl^- (again >80 % mortality). Larvae exposed to the Ag NPs at high Ca became noticeably lethargic with reduced swimming compared to controls. Increased $NaCl^-$ concentration resulted in increased survival with no significant differences between the two forms of Ag (ANOVA, $P > 0.05$). At 1 mmol l^{-1} $NaCl^-$ mortality was 68 and 60 % in Ag NP and $AgNO_3$ exposed larvae respectively, decreasing to 30 and 25 % in the presence of 10 mmol l^{-1} $NaCl^-$. Following exposure at pH 6, 7 or 8 there were no significant differences in acute toxicity between Ag NPs and $AgNO_3$ (ANOVA, $P > 0.05$), though both experienced increased mortality at pH 6 and 8 compared to pH 7 (ANOVA, $P < 0.05$). Increased concentrations of HA were seen to increase mortality in Ag NP exposed larvae (40 and 60 % at 1 and 10 mg l^{-1} HA respectively, ANOVA, $P < 0.05$), but was somewhat protective to $AgNO_3$ exposed animals. The general consensus is that there will likely be increased NP aggregation with increased HA, which could result in reduced exposure and thus toxicity. However, this was not seen here and further work is required to understand this effect. Whilst TiO_2 NPs and MWCNT were generally not affected by changes to water chemistry, significant effects were seen following exposure to Ag NPs.

Human exposure to emerging contaminants: monitoring and modeling (P)

MO181

Evaluation of Lead Contamination in a Shooting Range in Qatar

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Recreational shooting in indoor and outdoor firing ranges is becoming popular in Qatar where several natural factors, such as high salinity and low organic matter content can accelerate lead (Pb) pellet weathering and; thus, potentially contaminate the environment. However, little information is available as to the impacts of Pb pellets on the environment. Therefore, there is a need to determine Pb contamination levels at shooting ranges in Qatar. In this study, the concentrations of Pb in soils and dust collected from indoor, semi-outdoor, and outdoor ranges were evaluated. Lead was extracted from soil and dust samples using open acid digestion method, then its concentrations were determined using an ICP-MS. A baseline data on the health status of shooters was carried out using a 12-questions survey questionnaire. Results showed that maximum concentration of Pb in soil samples collected from outdoor ranges at 76 m was 148,557 ppb. When the Pb concentrations in dust samples collected from semi-outdoor ranges were compared between the shooting line and target line, it was found that the Pb levels were significantly higher ($p<0.05$) at the target site. The dust samples collected from 25 m indoor range contained relatively higher concentrations of Pb than those of 50 m indoor range results. Overall, the concentrations of Pb were determined to be significantly ($p<0.05$) higher in indoor ranges than those of outdoor and semi-outdoor ranges. These results indicate that there is a clear need for improving the situation in indoor ranges by using lead-free ammunition or by better ventilation. Based on the survey data, it was estimated that 25% and 15% of participants suffer from anemia and reduction in their mental capacity, respectively, based on self-report. This could be related to the recreational exposure to Pb, however, the cause and effect can only be established through controlled clinical studies.

MO182

Mass concentration and elemental composition of traffic-related particulate matters in Doha, Qatar

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Particles generated from vehicle exhausts negatively impact public health since they can deeply penetrate the respiratory tract carrying considerable amounts of toxins including trace metals and hydrocarbons on their surfaces. In a recent report published by the Qatar Statistics Authority, it was reported that the annual PM10 readings were five times that of the set limit (50 µg/m3). Since there is a direct correlation between particulate matters (PMs) and their adverse health effects on humans, there is an urgent need to monitor the levels of PMs generated by vehicles and identify their elemental composition to determine their potential health effects on Qatari population. The aim of this study was to determine the levels of traffic-related PMs and their elemental compositions in Doha, Qatar during February and March 2013. The PM2.5 and PM10 concentrations were measured using DPS monitors. The elemental composition of PM samples was determined by an ICP-MS. During the study period, it was determined that there is a direct relationship between the concentrations of elements detected in PM samples and the number of cars passed through sample collection sites. The environmental factors, such as temperature, humidity, and wind speed did not influence the concentrations of PMs. The levels of Pb, Ni, and Cr which are known carcinogenic metals were reported to be between 200 and 900 ppb, indicating a possible public health hazard. The presence of high concentrations of Al, Mg, Na, Fe, and Zn referred to the origin of PM to be mainly crustal material, sea salt, and fossil fuel combustion. The results obtained in this study might be helpful in establishing new air quality standards to protect the public health and the environment in Qatar.

MO183

The Nutritional-Toxicological Conflict Associated with Antarctic Krill Oil Dietary Supplements vs. Fish Oil Alternatives

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Fish are a nutrient-dense food source. The role of marine-derived, long-chain (LC) (≥C20) Omega-3 (ω3) fatty acids, in particular docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), in the promotion of health is well established. Accordingly a host of international agencies recommend consumption of fish at least twice a week. Modern diets in developed nations are however characterised by severe LC ω3 deficiency, reflecting low seafood intake. In fact, it must be considered that meeting health targets for seafood intake is not economically, nor ecologically attainable for large fractions of the global population. In the absence of sufficient high quality, affordable seafood sources, dietary supplements are pitched to play a role of increasing strategic importance. A new nutriceutical oil derived from Antarctic krill *(Euphausia superba)*, has recently been launched on the seafood-oil market. Marketing is based on three key promotional statements, namely; the improved bioavailability of krill oil ω3, the sustainability of krill fisheries and, finally, that krill are free of toxins and pollutants. Persistent Organic Pollutants (POPs) are ubiquitous, toxic and bioaccumulative contaminants and the vast majority of human exposure to POPs occurs via seafood consumption. In the case of fish oil dietary supplements, the situation is even more acute. Legacy POPs are lipophilic and accumulate in the lipid reserves of animals. When the lipid fractions of fish oils are selectively isolated and concentrated for administration as dietary supplements or complementary medicines, the fish oil micronutrient:POP burden conflict is exacerbated. Here we evaluated the nutritional quality and the contaminant burdens of Antarctic krill oil versus nine other fish oil dietary supplements readily available on the Australian market. All products adhered reasonably well to manufacturer DHA and EPA specifications and no products, at the maximum recommended dose, came close to fulfilling the contaminant tolerable daily intake (TDI) values assigned by international regulatory authorities. Contaminant profiles of Antarctic krill oil were distinct from other products, although not consistently lower. These results provide much needed quantitative data for a rapidly expanding new product in the fish oil market sector and in particular for the krill fishing industry.

MO184

Effects of cooking on the subcellular partitioning of Cd in cooked rice

B. Chen, H. Lai, C. Liu, MingDao University

Rice and rice products are staple foods in Asia. Rice grains may accumulate excess cadmium (Cd) when exposed to Cd-contaminated soil. Therefore, it is necessary to assess and manage the health risks of populations exposed to Cd through rice products consumption. In this study, two varieties of paddy rice were hydroponic cultivated in the Cd contained solutions to investigate their uptake and subcellular distribution of Cd. In addition, the subcellular partitioning of Cd in cooked rice under different cooking treatments was also examined. Experimental results showed that approximately 92-99% of the accumulated Cd was bounded to the cell wall and vacuole in the shoot and root of rice plants. The subcellular partition of Cd in cooked rice was significantly affected by different cooking treatments (boiled, steamed, and stir-fried). Generally, the proportion of Cd concentration in cell wall debris was increased with increasing heating duration and temperature of cooked rice. Results of the present study can be used to refine the exposure assessment in human health risk assessments.

MO185

Screening chemicals in commerce in the Nordic countries using multimedia fate and bioaccumulation models

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Increasingly, quantitative structure property relationships and/or environmental multimedia models are used to screen chemicals in commerce for substances that are potentially of concern for human health and the environment. These efforts have largely focused on hazard-related properties such as persistence, bioaccumulation, and long-range transport potential. This is partly because risk-based evaluations are often considerably limited by highly uncertain emission estimates, which render predicted concentrations and hence also chemical rankings uncertain. The availability of detailed information on the usage of hundreds of chemicals in commerce in Norway, Sweden, Finland, and Denmark for the years 2000-2007 through the SPIN (Substances in Preparations in Nordic Countries) database may allow for emission estimates with increased confidence within a Nordic context. Concentrations in the environment and in humans were calculated by inputting emissions, obtained by a combination of SPIN data and a high-throughput estimation method, into a dynamic integrated environmental fate and food chain bioaccumulation model (CoZMoMAN) specifically parameterized for the Nordic region. The chemical ranking based on those concentrations was then compared with those derived with a steady-state screening level risk assessment model (RAIDAR), parameterized for an evaluative environment and food-chain. This allowed us to investigate the impact of a steady-state assumption and a region-specific parameterization on the chemical ranking results.

MO186

Tox-Box: Developing a Test Battery for Toxicological Assessment of Anthropogenic Micropollutants in Drinking-water – A first Evaluation

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The joint project Tox-Box (BMBF 02WRS1282A) aims at evaluating anthropogenic (micro-)pollutants in terms of possible genotoxic, neurotoxic and endocrine disruptive effects. To establish a guideline value when limited data is available, the UBA developed a theoretical concept called “Health related indicator value (HRIV)”. This is the foundation of Tox-Box, which will define a test battery that provides in vitro data for turning a theoretical concept into a practical one. The tests used in Tox-Box will thus be aimed at creating in vitro data to allow the evaluation of the endpoints addressed in the HRIV concept. In the first part of the project single substances are tested in different test schemes, specific for the endpoints genotoxicity, neurotoxicity and endocrine disruption. Once these test schemes are established, substances having different effects on the endpoints tested can be distinguished. Based on the results, HRIV values for drinking water can be derived. The sub-project “In-vitro testing of neurotoxicity” tested 20 chemicals in a three-tiered approach. A first set of experiments employs the comparison of cytotoxic effects in cancer cell lines which had their origin in different organs, i.e. liver, blood or nervous system, to give a 1st hint towards possible neurotoxicity. Tier 2 uses a more thorough approach, when mitochondrial membrane potential, apoptosis, formation of reactive oxygen species and GSH content are tested. When the assumed neurotoxicity is substantiated, the 3rd rd tier is applied. Here the cytotoxic effects on primary astrocytes, activity of a neuron specific enzyme and neurite outgrowth are scrutinized. First experiments with acrylamide as a positive control indicate that primary cells are much more susceptible to this agent than the neuroblastoma cell line SH-SY5Y, which is used in the 1st and 2nd tier. Moreover, investigations in liver cancer and neuroblastoma cells clearly show a stronger acrylamide effect on the latter. So far our results indicate, that of the 20 substances tested, 7 have a neurotoxic potential. Among these is dichlorvos, which is a known inhibitor of acetyl cholinesterase, a neural transmitter. Dichlorvos was used as proof of principle. We are currently running the tests of the compounds selected for tier 3. Our results regarding dichlorvos were confirmed in the Tox-Box sub-project “In-vivo testing of neurotoxicity” which performed similar experiments in zebra fish (*Danio rerio*).

MO187

How to produce drinking water from surface water of the Three Gorges Reservoir in China according to the German drinking water directive?

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The impoundment of the Three Gorges Reservoir (TGR) in China is widely blamed and investigated for its impact on the ecosystem and water quality [1-3]. Water quality in the Yangtze river and its tributaries is therefore of major concern, as in the catchment area of the TGR surface water is commonly used as the raw water source for public water supply [4]. Additionally, it is considered for the provision of fresh water for the dryer northern part of China, as part of the south-north water diversion project [5]. Samples from surface water, tap water, and waste water treatment plant effluents in the TGR area were shown to contain mainly herbicides, trihalomethanes, and pharmaceuticals, respectively, in concentrations above the limit values given by German water directives [6]. In this study, therefore, the adsorption of the main pollutants clopyralid, picloram, chloroform, and bromoform in TGR surface water was studied on the three powdered activated carbons AquaSorb® BP2, AquaSorb® CP1, and ColorSorb® G9 (Jacobi) and the chelating resin AMBERLITE™ IRA743 (Rohm and Haas Industrial Processes).
Additionally, oxidation experiments with ozone and hydroxyl radicals were performed for herbicides (atrazine, clopyralid, and picloram), an antibiotic (sulfamethoxazole), and bromide. The results indicate a good adsorption behavior and wide range of oxidation of the analyzed compounds until a limitation by toxic by-products. Hence, the application of an activated carbon filter in drinking water treatment is a useful treatment step and an oxidation with ozone and hydroxyl radicals possible. This study is part of a Sino-German joint research project supported by the Federal Ministry of Education and Research (Germany) [7].
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MO188

Challenges for building a safety net for biocidal products: a Korean case

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Biocides are used in massive amount worldwidely for a variety of purposes. Due to their inherent activities on living creatures, the safe management of them is required for human and the environmental health. Unlike strictly regulated and managed pesticides, biocidal products still remains in a dead zone in terms of the safe management of chemicals in many places of the world. As a result of the uncontrolled safefy management of biocidal products, it was reported in 2011 that unidentified fatal lung disease found in Korea is suspected to have been caused by chemical disinfectants used with household humidifiers. The suspected active ingredients were found to be cationic synthetic oligomers that had been put in the water tanks of humidifiers for the prevention of germs, mold, and/or algae proliferation. Although, it is not very difficult to predict that this use of reactive cationic oligomers will end up nano-sized aerosols in the indoor environment, even a screening-level assessment was not been attempted. Immediately after this tragedic case, the Korean government has been trying to build a safety net for the regulation of biocidal products. However, the lack of information how and how much biocidal active ingredients are currently used in various products is now revealed to be a bottle-neck in the law-making process. The existing chemical regulations in the country are based mostly on the potential hazard of priority chemicals with high toxicity and production volume, but not on the use and the consequent risks of chemicals. Therefore, it is now immediately needed that the use of the biocides should be incorporated to a new chemical management framework. Due to the limited resources and time for building a safety net for biocides, a smart, prudent approach is needed. Herein, we propose both top-down and bottom-up approaches. In a top-down approach, the active ingredients that are used in a large quantity and already evaluated with respect to their chemical safety, the safety evaluation should be re-made by the governmental authorities for the specific uses of the activie ingredient. In the bottom-up approach, those who intend to sale biocidal products in the market should be enforced to register new biocidal products and the safety evaluation should be made for the specific uses. With both approaches, we expect that unexpected fatal accident due to the mis-use of biocidal active ingredients will be minimized in the future.

MO189

The HEALS approach to health and environment-wide associations

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1. Introduction The exposome represents the totality of exposures from conception onwards, simultaneously identifying, characterizing and quantifying the exogenous and endogenous exposures and modifiable risk factors that predispose to and predict diseases throughout a person’s life span. Unraveling the exposome implies that both environmental exposures and genetic variation are reliably measured simultaneously. **2. Materials and methods** The HEALS (Health and Environment-wide Associations based on Large population Surveys) approach brings together and organizes environmental, socio-economic, exposure, biomarker and health effect data; in addition, it includes all the procedures and computational sequences necessary for applying advanced bioinformatics coupling advanced data mining, biological and exposure modeling so as to ensure that environmental exposure-health associations are studied comprehensively. The overall approach will be verified in a series of population studies across Europe, tackling various levels of environmental exposure, age windows and gender differentiation of exposure, and socio-economic and genetic variability. The HEALS approach will be refined on the basis of **pre-existing population data** and then it will be applied in a **pilot environment and health examination survey** covering eighteen EU Member States. **3. Results and discussion** Exposome studies will require new tools to address the complexity of emerging environmental health issues. Critical for success will be the ability to bring together existing geospatial, environmental, health and socioeconomic data, and to collect new high resolution data using novel environmental micro-sensors, remote sensing or other community and omics/systems biology based approaches approaches to describe the exposome for e.g. endocrine disruption-related syndromes and sex-related changes (menopause), neurodegenerative or respiratory diseases. Focus will be on susceptibility windows during growth (including pregnancy) and development, and on the unequal distribution of the burden of epigenetically active food and environment-related disease to vulnerable populations such as the young, elderly, socio-economic disadvantaged, gender and ethnic minorities. **4. Conclusions** The lessons learned will be translated into scientific advice towards the development of **protocols** and **guidelines** for the setting up of a European environment and health examination survey.

MO190

The German Environmental Specimen Bank – Long-term Monitoring of Human Exposure and Exposure Factors

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The German Environmental Specimen Bank (ESB) is an important part of the German health-related environmental monitoring program. It is coordinated by the Federal Environment Agency (UBA) and monitors time trends in human exposures and changes in exposure relevant factors. The ESB collects, stores, and analyzes human samples since the 1980s. As a new member of the ESB, since 2012 Fraunhofer IBMT acquires blood, plasma and urine samples of approx. 600 young adults annually using a biological safety level 2 mobile laboratory. Several health-relevant pollutants and physiological parameters are quantified in these samples by initial real time monitoring. Besides, each volunteer fills in an extensive questionnaire on exposure factors and exposure relevant behaviors. Additional cryopreservation of samples allows for rapid access to samples collected over decades for retrospective analyses and trend evaluation. Results are evaluated and reported to the UBA annually. Emerging pollutants like plasticizers and synthetic compounds are currently discussed to have a diverse range of impacts on human health. Especially diethylhexylphthalate (DEHP) is still used in a wide variety of consumer and industrial products. Hence, human exposure to DEHP is omnipresent. Health related effects of DEHP are discussed to range from endocrine disruption, reproductive dysfunction to diabetes and cancer. Latest results of the German ESB document the success of regulatory efforts to reduce human exposure to phthalates like DEHP. In contrast, same data indicate an increase of other plasticizers (e.g., DINP) used to replace those classified as reproductive toxicants. Hence, within the German health-related environmental monitoring the ESB serves as an early-warning system regarding changes in human pollutant burden patterns

and exposure affecting characteristics of the population. Therefore, the continuously generated ESB data are vital for priority setting in population-representative human biomonitoring studies as well as maintaining up-to-dateness of German exposure factors as an important basis to generate exposure models for the general population. Moreover, the ESB is a highly flexible tool for assessing the success of policy measures aiming at reducing the human exposure to contaminants. Keywords: phthalates, human biomonitoring, mobile laboratory, environmental specimen bank

MO191

Detection of Aflatoxin M1 concentrations in human milk samples in Novi Sad, Serbia

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Aflatoxins, extracellular toxic metabolites produced by some fungal species from genus Aspergillus (A. flavus, A. parasiticus), are inducing aflatoxicoses, which are having toxic, immunosuppressive, theratogenic and cancerogenic effect. AM1 (i.e. milk toxin) is produced by human and animal metabolism in the liver, after eating food or feed contaminated with aflatoxin B1. During development of fetus, prenatal, it absorbs certain amount of toxins from mother organism. After birth, infant is through milk exposed to aflatoxin M1. AM1 in human milk samples was determined in Turkey, Egypt, Sudan, Kenya, Jordan, Iran and other countries; however there are no results on this topic for the region of East Europe and also for Serbia. After detection of AM1 in cow milk in Croatia and Serbia, urgent need is arisen to detect AM1 in human milk, to estimate status of whole environmental and health of newborns. Samples of colostrum were collected on Gynecological and obstetrical clinic of Faculty of Medicine in Novi Sad. Qualitative and quantitative determination of AM1 concentration level in human milk have been done with imuno-enzymatic tests kits, Imunolab Aflatoxin M1 (NOACK). Concentration of AM1 was determined photometric on 450nm on ELISA rider (Multiscan EX, Thermo Electron Corporation). Method sensitivity was lower than 10 pg/ml. A total of 33 samples (25 colostrum samples, 5 human milk samples and 3 commercial milk formulas) of whole milk were analyzed. Aflatoxin M1 residues were detected in 56% of colostrum samples. None of 3 commercial milk formulas contained AM1. In all human milk samples from mothers who breastfeeding babies 3 to 7 months older, AM1 was detected with the mean value of 247±176 pg/ml. Obtained concentrations levels of AM1 in samples show prenatal exposure of fetus, mothers and contamination of food products by aflatoxins. Monitoring results also give concentration profiles of AM1 in correlation of demographic parameters, such as mother's age, number of pregnancies and child births, occupation, body weight of newborn. Application of results gives special possibility to assess risk of fetus exposure and as well newborns on this mycotoxin, and also the first screening is of extraordinary importance to evaluate conditions of environmental contamination in Serbia. Acknowledgement: The research has been supported by Provincial Secretariat for Science and Technological Development of AP Vojvodina (114-451-3989/2013-03).

MO192

Body burden of POPs of Serbia residents, based on human milk and umbilical cord blood

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This initiated study is one of the very few investigating the body burdens of POPs in residents of Serbia. POPs pose a danger to the ecosystems and human health as they are bioaccumulative, resist, cycle in the environment over long periods, and can be transported over long distances. Especially susceptible might be the fetus, which is exposed in utero, and newborn breast-fed infant, since both are exposed to relatively high levels of POPs during a critical period of organ growth and development. Destruction during the NATO operation in former Yugoslavia caused the emission of POPs into the environment. No systematic and regular human biomonitoring of POPs has been performed in the West Balkan Countries till now. This study was carried out in collaborations with 5 Serbian national and research institutions. In order to gather reliable and comparable data, participants were encouraged to adhere as closely to WHO protocol as possible. In this study umbilical cord blood samples of healthy pregnant women aged between 20 and 42 years at the time they gave birth and human milk samples from the same women following delivery were collected during February 2013 from 100 participants living in Vojvodina. All samples were analyzed for their content of DDT, DDE, DDD, dioxin-like (PCB 118) and indicator (28, 52, 101, 138 153, 180) PCB congeners. Measurements were performed using GC ECD VARIAN CP-3380. Concentrations of PCBs and OCPs were in the range of 3-91 ng/$g_{lipid\ base}$ (med: 13, arith mean±stdev: 18.67±15.59), or 2-307 ng/$g_{lipid\ base}$ (med: 75.6, arith mean±stdev: 81.36±69.28), for the human milk, respectively. In all umbilical cord blood samples levels of analyzed POPs were under the LOD (< 0.5 ng/g, lipid adjusted). The four congeners (118, 138, 153, 180) contribute the main share to total body burden of PCBs. Higher chlorinated PCBs were found in up to 10-fold higher concentrations in human milk in relation to the lower congeners, while the levels of hexaCB were 3-4-fold higher than the levels of heptaCB. The body burden of POPs increases with age and decreases over the total nursing period. Women who had lived outside highly industrialized areas showed lower concentrations of PCBs. Birth weights didn't influence of POPs concentrations on human milk burden. New information on mother-child transfer by human milk and umbilical cord blood of POPs is needed, since it controls incentive of breastfeeding, indispensable way for health infant's development and growth.

MO193

Concentrations of phthalates metabolites in breast milk of Korea, 2011-2012: Current contamination, exposure amount, and risk assessment

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Phthalates have been used in a variety of consumer products and have been associated with endocrine disruption and developmental effects in many experimental and epidemiological studies. However, very limited information is available on phthalate exposure among infants through breast milk feeding. In order to understand the levels of phthalate exposure among breastfed infants, 132 breast milk samples were collected from breast feeding mothers in four cities of Korea in 2011-2012. Six phthalate metabolites, i.e. mono-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono(2-ethyl-hexyl) phthalate (MEHP), mono(2-ethyl-5-oxo-hexyl) phthalate (5-oxo-MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP), and monoethyl phthalate (MEP) were analyzed. MEP and MEHP were detected in 100 % of breast milk with median value of 0.35 and 2.47 μg/L, respectively. MiBP and MnBP were also detected > 94% of breast milk samples. However, the secondary metabolites of DEHP (5-oxo-MEHP and 5-OH-MEHP) were less frequently detected. The levels of metabolites among Korean mothers are far lower compared to those reported elsewhere. Demographic or seasonal characteristics could not explain the variations of the phthalate metabolites in breast milks, suggesting that exposure sources of phthalates vary among individuals. Median and 95th percentile daily phthalate intake through breast feeding was estimated at 0.35 μg/kg body weight (bw) and 2.22 μg/kg bw, respectively, for MEHP, 0.24 μg/kg bw and 3.09 μg/kg bw for MnBP, for an exclusively breast-fed infants (3 months old). The estimated phthalate metabolites intakes corresponds to only about 2% (median) to 51% (maximum) of reference dose of parent compounds.

MO194

Non-targeted screening of mercapturic acids in human urine – comparison of different LC-MS approaches

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Glutathione conjugation is an important detoxification pathway in the human body to eliminate reactive electrophiles formed by metabolisation of internal biomolecules or pollutants taken up from diet or atmosphere. Among other pathways gluthathione conjugates are subsequently converted to mercapturic acids and renally excreted. Thus, they can be used as biomarkers to assess the electrophilic burden of the human body reflecting both the internal and external chemical environment. This study aimed at developing and comparing two analytical approaches to detect mercapturic acids in urine in a non-targeted fashion. Both methods are based on the fact that all mercapturic acids show a common neutral loss during mass spectrometric fragmentation. Using LC-MS techniques, two different neutral loss scan methods were developed. An in source fragmentation scan was performed on a high resolution ion trap-Orbitrap mass spectrometer followed by a software-based search for the exact neutral loss mass difference. A QTrap mass spectrometer in constant neutral loss scan mode at unit resolution was used for a rather fast screening method. For the evaluation of both methods, a small set of spiked and native urine samples was analyzed. Both methods were compared regarding sensitivity and selectivity, their potential for structure elucidation of the unknown mercapturic acids and the capability to enhance sample throughput.

MO195

PFAS concentrations in cat serum and association to thyroxin levels

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An analytical method was developed and applied for the analysis of fifteen per- and polyfluoroalkyl substances (PFASs) in blood serum. The method is fast, demands a small sample volume (50 μl) and was successfully validated regarding recovery, accuracy and repeatability. The method consists of a methanol precipitation and an online SPE clean up of the whole sample. Chemical analyses were carried out using HPLC-MS/MS measurements with multiple reaction monitoring. Internal standard recoveries varied among PFASs (39–84%) and median recovery were 60%. This is on the low end, and probably related to the loss of sample material due to induced protein precipitation during sample preparation. Here it is regarded sufficient for accurate level concentrations. The accuracy was 55–110% for the PFASs and the method repeatability standard deviation was 1–7%. The limits of detection (LODs) were between 3 and 50 pg/mL depending on the congener. The method was applied to serum samples (n=30) from cats in Swedish households sampled between 2010 and 2011, within the framework of the recently started project MiSSE (Mixture Assessment of EDCs, Formas 210-2012-131, www.mmk.su.se/misse/). The project aims to use cats as models for indoor exposure to dust related endocrine disruptors, also relevant for humans and especially toddlers. The cats were suspected or known to have feline hyperthyroidism, a disorder also common in humans, hence the thyroxine (T_4) have been measured and reported. Some cats were treated with thiamazole orally. The PFAS profile was dominated by PFOS (median 2.8 ng/g fw), followed by PFOA (median 1.5 ng/g fw). The concentrations were slightly lower or similar to human levels, except the long-chain PFASs (C9-14) which were elevated. The T_4 levels and PFASs serum concentrations in corresponding samples were tested for linear correlation. A negative linear correlation between T_4 levels and long-chain (>C9) PFASs in cat serum could be observed. The cause for this is not yet fully understood. It can be an expression of the higher metabolism correlating to higher T_4 levels. The determined binding affinity of PFAS to the T4-transport protein transthyretin did not correlate to the concentration of the long-chained PFASs. No correlation between shorter chain length and T_4 could be observed.

MO196

Total intake estimates of PBDEs derived from PBDE biomonitoring data sets from the Australian population

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In Australia, polybrominated diphenyl ethers (PBDEs) have been measured in large-scale biomonitoring studies in 2-year intervals since 2002/3. Since the technical mixtures of penta-BDE and octa-BDE were banned by the Stockholm Convention on Persistent Organic Pollutants only in 2004, the biomonitoring data has been collected simultaneously with the ban. Here we use a population pharmacokinetic model to back-calculate PBDE intake by the Australian population from these biomonitoring data. When solved in a forward way, the pharmacokinetic model calculates the PBDE body burden of individuals born in different years on the basis of PBDE intake data and intrinsic elimination half-lives of individual PBDE congeners. Here we use the model in a backward calculation that adjusts the PBDE intake in such a way that the body burden calculated by the model is as close as possible to the PBDE biomonitoring data; the elimination half-lives of the PBDEs have to be provided as model input parameters. We investigate the most common PBDE congeners (i.e. BDE-47, -99, -100, -153); as model input we use four cross-sectional biomonitoring studies conducted in 2002/3, 2004/5, 2006/7, and 2008/9 in Australia and the intrinsic elimination half-lives of 1.4, 0.8, 1.8, and 7.4 years for BDE-47, -99, -100, and -153, respectively. The back-calculation of the intakes resulted in an adult intake of BDE-47, -99, -100, and -153 of 3.0, 2.0, 0.6, and 0.4 ng/kg/d, respectively. These estimated intake rates are about a factor of 10 higher than what is expected when the intake is calculated in a bottom-up exposure assessment, i.e. derived from PBDE concentrations found in indoor media (dust and indoor air) and diet, multiplied by the default contact rates. As has also been shown for the US population, to explain the measured body burdens, the PBDE exposure has to be much higher in Australia than what is calculated in bottom-up exposure assessments.

MO197

Measuring Persistent Organic Pollutants in Newborn Blood Spots: Performance and Stability of Brominated Flame Retardants, PCBs, and Halogenated Pesticides

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Archived newborn blood spots (NBS) can provide accurate and precise estimates of prenatal exposures to environmental toxicants. While several literature reports and pilot studies have used blood spots from human and avian samples to measure persistent organic pollutants (POPs), broad application of this approach for quantitative exposure estimation purposes requires methods that are robust and validated. Specific quality assurance needs include assessments of storage stability, background contamination, recovery and variability; also needed are reference materials, control samples and other QA elements. These concerns are especially significant for organic toxicants measured on archived NBS given the potential for gradual loss of sample integrity over multi-year periods. The goal of this study is to evaluate that methods for measuring persistent organic pollutants (POPs) in newborn blood spots (NBS) are valid and meet quality assurance goals. A laboratory study was conducted to evaluate three groups of POPs: brominated flame retardants (BFRs), e.g., polybrominated diphenyl ethers (PBDEs); chlorinated pesticides, including both legacy contaminants such as DDT as well as currently used but restricted pesticides; and polychlorinated biphenyls (PCBs). Each of these groups includes toxicants that have been found in human blood at high levels. While the use of many POPs is now restricted, past use in residential, commercial and industrial applications were extensive and body burdens remain elevated. Methods were developed to prepare, store, extract and analyze the bloodspots. Sample integrity was evaluated as a function of storage time and storage temperature .At -20 ° and -80 ° C, results showed negligible or only very slowly declining concentration trends and the absence of decomposition products, suggesting that sample integrity of POPs in blood spots remained high, i.e., losses are low. This applied to 16 PCB congeners, 18 BDE congeners, and most of the halogenated pesticides. However, storage at room temperature (20 °C) did not maintain sample integrity for several compounds, e.g., 5 PCB congeners, several pesticides and 6 BDE congeners, and the appearance of hydroxy-BDE at temperatures suggests enzymatic breakdown. With appropriate storage conditions and possibly with temporal correction factors, these results suggest that NBS can be used to estimate historical exposures to many POPs.

MO198

Risk analysis methodology principles and criteria

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The most important scientific and practical tools to support informed decision-making on environmental management for human health protection involve principles and criteria of modern methodology for risk analysis. The risk analysis procedure consists of such elements as risk assessment, risk management and risk communication. The following issues should be also considered: - hazard identification, - assessment of "exposure (dose, concentration)-response" dependence; - exposure assessment; - risk characterization; - comparative analysis and ranking of risks; - defining levels of acceptable risk; - principles for selection of strategies to reduce and control the risk; - options of management (regulatory) decision making; - basic features of risk perception and risk communication among specialists of risk assessment, risk governance officers, the mass-media, interested and concerned groups of stakeholders and the general public. Chemicals present in the environment and potentially influencing the population should be revealed at the stage of hazard identification. This stage of risk assessment is of screening nature and presupposes: 1) identification of environmental pollution sources and the probable effects to humans; 2) identification of pollutants; 3) characterization of probably harmful effects of chemicals; 4) identification of priority chemicals for their further studies, including exposure routes and pathways for penetration into human organism; 5) revealing harmful effects probably caused by priority substances at the assessed exposure routes, duration (acute, sub-acute, chronic and life-long), as well as pathways of penetration into human organism (inhalation, oral, dermal). Implementation of risk assessment methodology in the system of environmental and human health quality management allows to: - Develop mechanisms and strategy of the various regulatory measures on risk reduction;\n - Obtain quantitative characteristics of health damage from exposure to harmful environmental factors;\n - Compare and rank different effects according to severity of the human habitat impact factors;\n - Reduce the uncertainty of analysis in decision-making;\n - Establish more reliable and safe exposure levels and hygienic standards;\n - Identify environmental policy priorities and human health protection policy. Keywords: risk analysis, environmental pollution, human health protection, hazard identification.

MO199

Investigation and monitoring of micropollutants in India and UK rivers by long term survey

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Micropollutants becoming one of the major concerns in natural waters as the results of their continuous release into the environment [1, 2], due to their wide use and consumption in modern societies. Micropollutants consist of various chemical groups among them most important are: Pharmaceutical and Personal Care Products (PPCPs) and Endocrine Disrupting Compounds (EDCs). Although many

of PPCPs do not exhibit bioactivity in the environment; but it has been well documented that aquatic fauna are at major risk of exposure from some of these compounds *i.e*. hormonal disruption in wild fish [3] caused by estrogenic hormones. It must be underlined major of the data and studies has been performed in the developed industrialised countries like USA or in Western Europe. Countries with rapid economic, urban and industrial growth (*i.e*. India), with little regulation of chemicals or treatment of wastewaters still lack of valuable information about the extent of micropollutants in natural environment. Recent EU legislation - Water Framework Directive (WFD) [4] - requires investigation, monitoring and assessment of pollution threat to protect natural waters. Directive has already identified 33 priority and 8 other pollutants for which there are environmental quality standards that must not be exceeded in freshwater bodies. A further 15 pollutants are under consideration, including pharmaceuticals e.g. 17 α-ethinyl estradiol and diclofenac. However, other programs are lunched *i.e*. The Chemical Investigation Programme (CIP) in UK [5] - to cover wider range of chemicals and provide vital data for risk assessment and perspective regulatory standards. The aim of this study was to estimate longer term extent of pollution (started July 2012) by selected micropollutants in two major rivers in India; the River Ganges and the River Yamuna. Those rivers are very important from cultural and economic aspects, both of which are also the subject of important governmental plans to improve water quality.

MO200

PBDEs in handwipe samples of mother and infant pair, and the associations with house dust

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PBDEs have been widely used as flame retardants, and are known to be persistent and potentially endocrine-disrupting. Infants are identified as one of the most vulnerable populations to PBDEs. Recent studies pointed out that one of the major sources of PBDEs is house dust. To identify potential exposure to PBDE among infants, we recruited 33 mother and infant pairs between July and August of 2013 in Seoul Korea, and measured the levels of several PBDEs in both handwipes and house dusts. A questionnaire was conducted for demographic characteristics, use of electronics, and others. PBDEs were detected in 81% of baby handwipes, 67% of mother handwipes, and 92% of house dust samples. Geometric mean of total PBDEs were similar in handwipes (1.8 and 2.4 ng/hand for infant and mother, respectively). Considering smaller hand surface of infant, the levels per unit hand area are higher in infant handwipes. The maximum levels of total PBDEs were 913.8 and 81.2 ng/hand in infant and mother handwipes, respectively. BDE209 was the most abundant congener in both handwipes and house dust, and the detection pattern of different congeners was similar, suggesting similar sources. However, PBDEs levels in hands of infant and mother are not significantly associated. Differences in activity pattern, e.g., more frequent contact with house dust, and less frequent hand-wash among infants may explain greater extent of PBDE exposure among infants. Exposure to PBDEs through hand-to-mouth activity may be a significant among infants and warrants further investigation.

MO201

Harmful organic chemicals in children's toys due to use of recycled materials

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Most of the materials used in consumer goods contain a number of additives which are meant to improve key properties like plasticity or flame resistance. At the end-of-life of the product, many polymeric materials are recycled and the additives they contain, such as flame retardants (FRs) and plasticizers, are transferred to the newly manufactured goods. We have investigated the occurrence and profiles of FRs, such as polybrominated diphenyl ethers (PBDEs) and phosphate FR (PFRs) and of plasticisers, such as phthalate esters, in 106 toy samples. For this purpose, material-specific extraction procedures were employed followed by GC-MS analysis using electron ionisation (for PFRs and phthalates) and negative chemical ionisation (for PBDEs). Low levels and detection frequencies of components of the technical Penta-BDE and Deca-BDE mixture were found, with BDE 209 being the dominant PBDE in all samples (maximum value was 0.14 mg/g or 0.014%). The levels of PFRs and phthalates were up to 10,000 times higher than those of the PBDEs, with triphenyl phosphate and diethylhexyl phthalate being the major representatives of these classes. Maximum values were 1.3 and 6.9%, respectively. The detection frequencies were up to 50% for PFRs and 98% for phthalates. All but one of the toys produced after the REACH regulation went into force complied with its provisions. The samples were grouped according to relevant selection criteria to assess the risk for children of different age groups. Using models in the literature, the exposure to these chemicals was tentatively assessed. It is clear that at the levels found in the investigated toys, these additives do not contribute to the intended characteristics of the materials, but may pose a health hazard to the children. Most likely, recycled materials are an important source of these additives in toys and therefore, their (re)use in products for children should be banned. As some of these additives are persistent and bioaccumulative in the human body, this is an additional cause for concern.

MO202

Examination of Diffusion of Plasticizers and Flame Retardants from Plastic Surface of Home Appliances and Constructions Directly into House Dust

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Plastic additives containing into plastic parts of home appliances not only evaporates into the indoor air but also diffuses directly into house dust attached to the plastic surface. However, it is very difficult to estimate the amount of diffusion of plastic additives from the plastic surface, because there is no appropriate method to measure the rate of diffusion of chemicals directly into house dust. Thus, we considered the experiment that uses glass beads in place of house dust, measured the rate of diffusion of plasticizers, and examined the estimation method of the amount of diffusion of chemicals. First, we prepared the PVC sheet samples including Bis(2-ethylhexyl) phthalate (DEHP) as a plasticizer, the HIPS sheet samples including Decabromodiphenyl ether (decaBDE) and the PC/ABS sheet samples including bisphenol A bis(diphenyl phosphate) (BDP) as flame retardants. Next, we placed the given amount of glass beads on the surface of these plastic samples and shook them to promote diffusion of chemicals into glass beads during the given period of time. Then, we measured the amount of diffusion by analyzing collected chemicals from the surface of the beads using TD-GC/MS or HP/LC. Finally, we calculated the rate of diffusion by dividing the amount of diffusion by the surface area of plastic samples and the given period of time. As a result, we found that the rates of diffusion of DEHP, decaBDE and BDP into glass beads were from 0.013 to 0.22, from 0.0002 to 0.0024 and from 0.001 to 0.015 mg/m2/h which were inversely proportional to the time of examination. We also found that the diffusion rate of those chemicals into glass beads is ten times faster than that into the air. Therefore, we consider that the major exposure route of plastic additives in indoor environment is diffusion of chemicals from the surface of plastic directly into house dust, and we will establish an experiment condition applying to other chemicals of plastic additives.

MO203

Environmental Contaminant Concentrations in Canada Goose (Branta Canadensis) Muscle: Estimation of risk to human consumers

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Not having access to sufficient food and food resources affects millions of people in the United States each year. To help these people in need there is a network of soup kitchens, food banks, and shelter that provide meals. However, often these organizations which rely on donations have limited food resources, specifically meat. To address this issue, USDA Wildlife Services donates more than 60 tons of wild game (deer, moose, feral hogs, goats, geese and ducks) to a variety of charitable organizations each year. Although commercially produced meat routinely undergoes screening for contaminants, the potential exposure to environmental contaminants from eating wild game is not well characterized. This study examined the concentration of 17 contaminants of concern in the breast meat of wild geese. With these residue data a probabilistic model was used to estimate potential risk associated with meat consumption. Based on model predictions, more than 99% of all adults were below exposure limits for all of the compounds tested. For all age classes modeled consuming goose meat may expose a small fraction of the populations to levels of lead greater than exposure limits. Similarly, mercury exposure was predicted to be greater than recommended limits when the meat was served in some of the meat preparations modelled. This study provides information about concentrations of contaminants of concern in goose meat harvested during the molting period and potential exposures associated with meat consumption using the probabilistic modelling. This information can enabling others to make informed decisions about the risks associated with meat consumption.

MO204

Occupational exposure of cashiers to Bisphenol A via thermal printing paper

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Bisphenol A (BPA) is the subject of debates all around the world due to its possible harmful effects in humans even at very low exposure levels. Increasing research efforts are being undertaken to further investigate exposure levels and pathways of

BPA. Although the use of BPA in consumer products gets a lot of attention, the focus of the work presented here is on occupational exposure to BPA, and more specifically on the potential dermal and oral exposure of workers (such as cashiers) via thermal printing paper (e.g., cash receipts). Cash receipts have been reported to contain BPA levels up to 3.2% w/w. Moreover, recent experimental work has indicated that BPA is easily transferred to the skin by touching BPA-containing thermal printing paper and modeling work has reported estimated daily intakes via cash receipts with typical values around or slightly lower than 1 μg/kg bw/day. No occupational exposure scenario was however included for this in the EU Risk Assessment Report on BPA (2003, addendum: 2008). In this study, a brand new scenario was developed for estimating daily intake of BPA via the skin through frequent contact with thermal printing paper in cashiers. The obtained results were compared to those reported in literature. Further, it has been evaluated whether or not oral exposure (which is normally not taken into account as a relevant route of exposure in workers) could be expected to be relevant for employees using BPA-containing thermal printing paper, and what would be the requirements for building an exposure scenario for this. Overall, it is generally accepted that food constitutes the most important source of BPA intake for the general population (> 90%). In this study it is evaluated whether dermal and/or oral intake of BPA through contact with thermal printing paper in cashiers contributes significantly to the total intake or not. It is generally accepted that uptake via the skin results in bypassing first-pass metabolism of BPA, which may significantly affect toxic unconjugated BPA levels in the blood, compared to when only oral intake would occur. This is taken into consideration in this project.

MO205

Skin bioavailability modelling for risk assessment: Key challenges related to skin sensitizing ingredients

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Assessing the risk associated with dermal absorption of chemicals is a crucial aspect of assuring the safey of many personal care products. A key scientific challenge is to develop new non-animal approaches for skin sensitisation risk assessment. To address this challenge it is necessary to consider how the physicochemical properties of a chemical influence the processes that control the bioavailability of a chemical ingredient. Such properties can be subdivided into different categories. For instance, in physical chemistry, properties of a chemical that are system independent are referred to as "intensive", whereas system dependent properties are referred to as "extensive". This seemingly esoteric difference has considerable implications for the use of chemical and toxicological information, particularly when attempting to better understand the mechanistic behaviour of chemicals with respect to skin bioavailability. Briefly, an intensive property is one that depends on the nature of the substance, not its quantity. An extensive property, on the other hand, depends on the amount of chemical present. In the context of allergic contact dermitis (ACD), whereby the ability of a chemical to act as a sensitiser is an extensive property of the chemical. The extensive property of dose or mass of sensitiser applied per unit area of skin is established as the critical dose-metric that determines whether an individual is sensitised. The objective of this study is to examine the question of how the intensive versus extensive nature of the properties of chemicals influence their skin bioavailability in both causing and modulating the immunostimulatory events that lead to sensitisation and ACD.

MO206

Persistent Organic Pollutants in males in the Tromsø Study 1979-2007

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Introduction: Human exposure to both newer and legacy persistent organic pollutants (POPs) has changed during the last century. Emissions of different POPs have changed over time, and the exposure routes have been through diet for the legacy POPs, whereas other routes have also been important for newer POPs. The legacy POPs were often observed to increase with age in cross-sectional studies and this association was likely reflecting birth-cohort differences in duration and intensity of exposure to these compounds. For newer POPs, conclusions of associations to age have not been consistent. Repeated measurements of individuals offer insight into changes with age, calendar time or birth cohort. **Methods:** The present study presents five repeated serum samples from 53 men in the period 1979-2008 analyzed for a variety of POPs (PCBs, organochlorine pesticides, brominated flame retardants, and fluorinated substances). These archived samples originate from the Troms ص Study, which is a population-based health survey in Troms ص, Northern Norway. **Results:** The summed concentrations of PCBs and pesticides decreased by 22%, 52%, 54%, and 68% from 1979 to 1986, 1994, 2001, and 2007, respectively. Whereas for the fluorinated substances, the median summed PFAS burdens increased 5-fold from 1979 to 2001 and decreased by 21% from 2001 to 2007. Thus, the composition of POPs in serum has changed over these almost thirty years. Brominated substances constituted little of the POPs burden relative to the other POPs analysed. The assessments of age, calendar time and birth cohort effects showed that calendar time was the dominating influence. **Conclusions:** The present study provided valuable insight to the time-variant exposure to many POPs for a group of subjects in Northern Norway. The observed trends during 1979 to 2007 likely reflect the overall trends in use and emissions of the different POPs, and the serum burden of the POPs analysed increased to 2001 and decreased to 2007. Trends for POPs likely differ depending on their changing emissions in combination with different persistencies in sources, environment and humans.

MO207

Application of metallic profiling of teeth for Environmental and Forensic Science

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It is well established that exposure to metals via skin contact, ingestion or inhalation leads to incorporation into calciferous material such as teeth and bone. Teeth are an ideal site to study the exposure of trace metals since they are the hardest structure in the body and preserve metals longer than soft tissues and bones. Both animal and human teeth have the potential to be used as a rich source for placement and tracking of pollution for environmental science, as well as being used to infer dental work, investigate both geographical variation, and to monitor nutritional status specific for forensic science. The composition of teeth changes over a significantly slower time period than soft tissues and blood levels in response to dietary and environmental uptake. In doing so this biomaterial can provide a permanent, cumulative chronological record at the time of exposure, providing a useful archive which may be used as an elemental fingerprint for both environmental and forensic science. The potential to identify and apportion the sources of metals are central to determining the practicality of these methods when applied to environmental and forensic science, while advancements in trace elemental analysis will continue to enhance research and development. Given the complexity of the matrix and potential problems associated with digestion and analysis methodology, a pilot study has been initiated using non human sources of sample to optimise the approach (rat teeth samples donated from another research project within UWS). The study acts as a prerequisite enabling the methodology to be validated on a similar structure and composition matrix. In this study, we used lower left and right mandibular incisors, which continually erupt and contain all stages of tooth formation. A comparison of the suitability of digestion methods applied to various reference matrices (organic and inorganic) will be presented. Destructive and non-destructive tests were performed on the teeth via inductively coupled plasma mass spectrometry (ICP-MS) and scanning electron microscopy (SEM). SEM was used to assess the feasibility for surface analysis by defining the transition effects between the root to the crown and ultimately the effect this has when trying to obtain a permanent time specific record of trace exposure. ICP-MS was used to infer the cumulative trace metal levels, including stable isotope analysis.

Advancing Adverse Outcome Pathways for Integrated Toxicology and Regulatory Applications (P)

MO208

Identification of research priorities for development of adverse outcome pathways in ecotoxicology

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Here we present a summary of workgroup activities within the March 2014 workshop on adverse outcome pathways (AOPs). To date, environmental risk assessment has relied heavily on whole-animal toxicity testing with direct observation of apical endpoints such as growth, survival or reproduction. However, such tests provide little information about chemical mode of action. This drives the efforts to develop alternative testing strategies. Further, a number of sublethal effects of toxicants on the organism with a potentially high significance for population-level impacts appear to be missed by the testing guidelines and recommended strategies most often applied today. Because mechanisms of chronic toxicity and effects of long-term exposure to low levels of potent micropollutants are poorly understood, current approaches to derive chronic safety values from acute toxicity data often lack a sound scientific basis. Development of AOPs covering subtle effects of contaminants on the endpoints other than growth and reproduction, would help identifying critical knowledge gaps to be filled in order to

understand the key events leading to manifestation of a certain whole-organism effect. Combined with further advances in the modeling and understanding of population dynamics in the wild, this would help predicting the impacts of sublethal effects of toxicants on population viability. The workgroup's objective was therefore to first describe generalized criteria for prioritizing AOP development, and then apply those criteria to suggest specific, near term, AOP development priorities, in particular for non-human taxa. Some specific topics and question to be tackled included (I) identification of critical areas in need of development of alternative testing strategies; (II) identification of research priorities for development of AOPs between and across multiple taxa to facilitate the reciprocal usage of data obtained in commonly used toxicity and ecotoxicity tests; (III) development of AOPs that characterize chronic toxicity and adverse outcomes of sublethal effects for which guideline toxicity tests are currently lacking or are highly challenging to perform, e.g. how to use the AOP framework to organize the information on the sublethal effects arising during chronic exposure? Development of AOPs for sublethal toxicity is expected to help streamline the research efforts aimed at developing novel tests with improved prediction potential for chronic toxicity outcomes.

MO209

Strategic approaches to adverse outcome pathway development

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Adverse outcome pathways (AOPs) are conceptual frameworks for organizing biological and toxicological knowledge in a manner that supports extrapolation of data pertaining to the initiation or early progression of toxicity to an apical adverse outcome that occurs at a level of organization relevant to risk assessment and/or regulatory decision-making. In order to support a more predictive paradigm in regulatory toxicology, organizations like the Organization for Economic Cooperation and Development (OECD), have initiated efforts to develop and disseminate AOP knowledge within the scientific community. While guidance on describing and evaluating AOPs has been provided, to date, generalized strategies for rapidly and systematically synthesizing available scientific evidence and knowledge into relevant AOPs are lacking. This presentation will report on results from an expert working group convened as part of an International Workshop on Advancing AOPs for Integrated Toxicology and Regulatory Applications to be held March 2-7, 2014. The workgroup will take the example of AOP development related to adverse impacts on avian reproduction as a case study to explore different strategies for AOP development. In particular, the group aims to examine (1) the use of molecular screening data to identify relevant molecular targets/potential initiating events; (2) the relative utility of biological systems knowledge versus literature and data-mining approaches to key event identification; (3) particular challenges related to development of AOPs for less studied organism classes (e.g. birds, aquatic organisms). An overview of approaches that did and did not work and a set of general recommendations for other AOP developers will be provided. *The contents of this abstract neither constitute, nor necessarily reflect, official positions or policies of the authors' employers or institutions. *This abstract includes the workgroup co-chairs only – see the poster presentation for a list of all contributing co-authors.*

MO210

Applying AOPs to support Informed Approaches to Testing and Assessment

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In advancing their vision and strategy for toxicity testing in the 21st century, the US National Research Council recognized that pathway-based approaches relying on measures of initiation of toxicity, rather than apical toxicity outcomes, may never be suitable for all regulatory applications. Higher tier risk assessments requiring high levels of certainty may necessitate the use of more traditional toxicity testing approaches for the foreseeable future. Nonetheless, resource constraints and the need for timely decision-making require that regulatory toxicity testing become more cost-effective and efficient. One way to achieve that goal is to become more strategic in the application of testing resources and focus the greatest amount of resources on the chemicals/chemical classes exhibiting the highest hazard potentials, and focusing the testing on the most probable hazards, in the most vulnerable species. The goal of prioritizing and focusing testing resources is the basic principle behind many existing "tiered" testing strategies. In addition to supporting more predictive or extrapolation-based approaches to hazard assessment, AOPs also provide a foundation for the design of "Informed Approaches to Testing and Assessment" that can strategically deploy screening level analyses to effectively focus testing resources and progressively deploy more resource-intensive confirmatory/uncertainty-reducing assays as required by the needs of a risk assessment. AOPs are well suited to this approach by encompassing a series of measurable biological activities/events detected at varying levels of biological organization along the progression toward an adverse outcome. The present work report on the outcome of workgoup 5 of the workshop "Advancing Adverse Outcome Pathways for Integrated Toxicology and Regulatory Applications" that was held March 2-7, 2014 in Somma Lombardo, Italy. This workgroup considered, discussed, and provided examples of how AOPs can be used to develop "Informed Approaches to Testing and Assessment (IATA)" and outcomes of these discussions will be presented herein.

MO211

New toxicity outcome using a non-model fish species Largemouth bass (Micropterus salmoides)

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The increasing amount of pollutants released within the marine environment is a problem that, year after year, is going to augment the negative effects on marine organism. In recent years the decrease in fish populations has been attributed to the potential impact of compounds on ovarian development. In addition, the consumption of fish may have downstream impacts on other species and also the human population. Understanding the molecular events occurring during ovarian development and the effects of pollutants on these molecular events could help in preventing adverse outcomes in both fish and predator species. The advances in 'omics' technologies have provided us with a powerful toolset which is able to measure a vast amount of genes within an organism and is central to our approach to understanding ovarian development. We focus our efforts on the Largemouth bass (LMB) (*Mycropterus salmoides*) which is widely distributed along the USA east coast. They have an important economic value due to their popularity as a sport fish but they are also ecologically relevant due to their trophic position in their freshwater environment as apex predators. We derive a transcriptional dataset for seven stages of ovarian development, perinuclear (PN), cortical alveoli (CA), early vitellogenesis (eVtg), late vitellogenesis (lVtg), early oocyte maturation (eOM), late oocyte maturation (lOM) and ovulation (OV), and apply a dynamical network inference approach. This allows us to identify potential groups of genes which may be disrupted as a result of exposure to Apopka lake (Florida). We identify potential chemical candidates of importance and validate these in a laboratory setting. With the use of 'omics' technologies we were able to build a dynamical model representing the development of ovaries in a sentinel fish species. By mapping effects of a known polluted area we were able to identify modules which are affected at different stages of ovarian development. We hypothesize that some of the chemicals we highlight using the CTD database may be central to the observed effect. Further laboratory validation of these compounds will be necessary and is currently underway. Interestingly, a strong interaction between ribosome biogenesis and energy metabolism is observed in our model which is highly affected by exposure to polluted areas.

MO212

Elucidating potential adverse outcome pathways related to exposure to ionic silver and silver nanoparticles on the fathead minnow

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Nanoparticles are compounds of emerging concern with largely unknown risks for human and ecological health. It is crucial to evaluate their potential biological impact to prevent unintended adverse effects on human health and the environment.We analyzed the transcriptional effects of polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNPs) and silver nitrate ($AgNO_3$) on the fathead minnow (*Pimephales promelas*) to understand their potential toxicity and adverse outcomes. Fathead minnow females were exposed to either 4 μg/L of $AgNO_3$ or 70 μg/L of PVP-AgNPs for 96h. Microarray analyses were performed on liver and brain. Functional analysis identified potential toxicity pathways and molecular initiating events (MIEs) that were confirmed with functional assays. Data suggested that $AgNO_3$ and PVP-AgNPs had both common and distinct transcriptional effects. The nanoparticles were linked to neurotoxicity and oxidative stress, and identified as a dopamine receptor antagonist. Silver nitrate was also identified as a potential neurotoxicant and was confirmed as adrenergic and cannabinoid receptors antagonist.While silver nitrate and PVP-AgNPs were both potential neurotoxicants, they appeared to act through different MIEs.

MO213

Effects of BDE-209 contaminated sediments on zebrafish development and potential implications to human health

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Polybrominated diphenyl ethers are compounds widely used as flame-retardants, which are of increasing environmental concern due to their persistence, and potential adverse effects. This study had two objectives. First, we assessed if BDE-209 in sediment was bioavailable and bioaccumulated into zebrafish embryos. Secondly, we assessed the potential impact on human and environmental health of bioavailable BDE-209 using human *in vitro* cell assays and zebrafish embryos. Zebrafish were exposed from 4 hours to 8 days post-fertilization to sediments spiked with 12.5 mg/kg of BDE-209. Zebrafish larvae accumulated ten fold more BDE-209 than controls in unspiked sediment after 8 days. BDE-209 impacted expression of neurological pathways and altered behavior of larvae, although BDE-209 had no visible affect on thyroid function or motoneuron and neuromast development. Zebrafish data and *in silico* predictions suggested that BDE-209 would also interact with key human transcription factors and receptors. We therefore tested these predictions using mammalian *in vitro* assays. BDE-209 activated human aryl hydrocarbon receptor, peroxisome proliferator activating receptors, CF/b-cat, activator protein 1, Oct-MLP, and the estrogen receptor-related alpha (ERRα) receptor in cell-based assays. BDE-209 also inhibited human acetylcholinesterase activity. The observation that BDE-209 can be bioaccumulated from contaminated sediment highlights the need to consider this as a potential environmental exposure route. Once accumulated, our data also show that BDE-209 has the potential to cause impacts on both human and environmental health.

MO214

Transcriptional responses of white sturgeon (Acipenser transmontanus) following exposure to a model dioxin-like compound

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Exposure to dioxin-like compounds (DLCs) can cause adverse effects in fishes through activation of the aryl hydrocarbon receptor (AhR) pathway. While there is a host of information regarding the effects of DLCs to modern fishes such as salmonids or cyprinids, little is known about the specific molecular mode of action and resulting physiological impacts of DLCs to sturgeons or other ancient species of fishes. With the aim of identifying specific molecular toxicity initiating events as a basis for hypothesizing novel adverse outcome pathways for DLCs in fishes, white sturgeon (*Acipenser transmontanus*) were exposed to the model AhR agonist, beta-naphthoflavone (BNF), and transcriptional responses were evaluated by use of *Illumina* RNAseq after *de novo* assembly of a reference transcriptome. Abundances of greater than 2,000 transcripts were altered by at least 2-fold in livers of white sturgeon exposed to BNF compared to controls. Abundances of transcripts of genes known to be regulated by the AhR, including those encoding proteins that catalyse Phase I, II, and III metabolism of xenobiotics, were greater in livers from sturgeon exposed to BNF. In addition, abundances of transcripts of genes from pathways not known to be involved in activation of the AhR were significantly up- or down-regulated. For example, abundances of transcripts of genes involved in responses to low concentrations of oxygen, such as aryl hydrocarbon receptor nuclear translocator (ARNT) and hypoxia-inducible factor 1 alpha (HIF1a), were lesser in livers from sturgeon exposed to BNF. This could be indicative of potential synergistic effects of sturgeon co-exposure to DLCs and hypoxia. Overall, next-generation sequencing technologies, such as *Illumina*, could prove useful in the discovery of novel biological responses to contaminants in non-model species of concern.

MO215

An updated version of H295R steroidogenesis system to assess chemical induced effects--- Integration of genes knockdown cell model, RNA-amplicon sequencing and LC-MS/MS quantification

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The H295R cell-based steroidogenesis assay is a well- established test system to assess the adverse effects of endocrine disrupting chemicals on steroid biosynthesis with mechanistic information. With the well-validated quantification capability on mRNA expression by RT-PCR method and on hormone measurement by ELISA, this method has been accepted by national and international organizations for chemical testing and management. To further improve the data quality generated by the assay and to expand it application in the assessment of environmental samples, here we present an updated version of H295R steroidogenesis system to assess chemical by integrating genes knockdown cell model, RNA-amplicon sequencing and LC-MS/MS quantification. The H295R cells can express all the necessary enzymes involved in steroidogenesis and thus have been widely used in research on steroid biosynthesis. To understand the effect of enzyme-specific loss of function on cell viability and steroidgenesis fuction, a knockdown model was developed, in which H295R cells were stably transfected with shRNA plasmid DNA to knockdown steroidogenic genes. 10 steroidogenic genes, such as CYP17A1, CYP19A1, NR1I2 and NR1I3, were knockdown individually, and 10 types of transfected H295R cell lines were performed. Then culture medium of each stable transfected cell line was extracted and the concentrations of steroid hormones were detected by LC-MS/MS. Comparison of responses with wide-type cells was used to elucidate the effect of gene specific knockdown on steroidgenesis. To validate the new system on the assessment of endocrine disrupting potential effect of chemicals and environmental mixture, ten water extract samples collected from Australia together with model chemicals (Forskolin and Prochloraz) were used to dose wide-type and knockdown H295R cells respectively. The assessment strategy was as follows: Firstly, RNA-amplicon sequencing technology was used to quantify the gene expression of targeted molecular pathways, including steroidogenesis pathway and stress responsive pathway. Secondly, LC-MS/MS was used to detect the concentrations of steroid hormones in culture medium. Finally, effect of environmental mixture and chemicals on steroidgenesis and stress responsive pathways was evaluated.

Delving into the social and monetarised environmental impacts during the evaluation process of the Life Cycle of products in order to be able to take all three pillars of sustainability into account (P)

MO216

Site- and operation-dependent external costs: an argument for less strict regulatory emission limit values for industrial installations?

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In the European Union (EU), emissions from industrial installations are largely addressed by requiring that best available techniques (BAT) are implemented. The current Industrial Emissions Directive (2010/75/EU) sets stricter emission limit values (ELVs) for existing combustion plants to be respected from 2016 onwards than previous regulation. As a new feature and under certain conditions, plant operators can apply for less strict ELVs on the basis of the disproportionate cost principle, comparing environmental benefits to (private) costs (Art. 15-4). This study aims to present a way how to quantify environmental benefits related to reductions in air pollutant emissions in economic terms. Further, the extent to which the proportionality of abatement options depends on different environmental and technical settings is assessed. The case is made for a DeNOx retrofit at a typical coal-fired power plant at varied Western European locations. Environmental benefits are quantified with help of a state-of-the-art tool for point sources in Europe: EcoSenseWeb version 1.3. Through a cost-benefit analysis, private costs of installing and operating the BAT at a given industrial site are confronted with associated monetised environmental benefits. For the default scenario, the DeNOx retrofit studied can be generally assessed to be efficient, i.e. the disproportionate cost criterion does not apply. Nonetheless, quantified environmental benefits substantially change according to the environmental setting. When varying technical parameters, e.g. reducing the operation time per year, the DeNOx retrofit may become disproportionate. This study shows that the results are sensitive to the environmental setting and key technical assumptions (e.g. full load hours, abatement costs). Besides, the results are subject to methodological assumptions (e.g. discount rate, particle toxicity) and further limitations like insufficient coverage of impacts (e.g. on ecosystems and biodiversity) as well as consistency issues such as different reference years (e.g. meteorological data, receptor data or studies on monetary values), varying aspects of the effects that are valued (e.g. resource vs. opportunity vs. disutility costs), different degrees of geographical validity (e.g. monetary values or risk functions), and different models for assessing concentrations of SO_2 and other classical air pollutants. Further methodological development is needed to make the assessment more robust.

MO217

Development of country specific weighting factors and estimation of external cost for G20 countries in LIME3

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LIME, an advanced life cycle impact assessment method based on endpoint modeling, has been developed as part of the LCA national project of Japan. One of the aims of LIME is to develop the weighting methodology, which enable us to integrate various environmental impacts that are used for life-cycle impact assessment (LCIA) and facilitates the interpretation of environmental information, such as in the selection of products. This project has now reached to the third phase with the additional aims, one of which is to update the weighting factors from the national average (LIME2) to the global scale (LIME3). We conducted the survey using same questionnaire in 19 countries to estimate country specific weighting factors. We report our results obtained by the main survey and calculated weighting

factors for G20 countries. The results can be used to develop integration factors in LIME3, enabling us to express LCIA results as a single index, such as external cost.

MO218
Comparing IMPACT World+ with other LCIA methodologies at end-point level using the Stepwise weighting factors
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The aim of the present paper is to test the use of economic weighting factors to put in perspective and compare IMPACT World+ damages with other existing life cycle impact assessment (LCIA) methodologies.The impact assessment methods Ecoindicator99 (H), Stepwise2006 and ReCiPe2008 (H) and IMPACT World+ were compared with respect to the relative and absolute importance that they assign to the different midpoint impact categories. Starting from the global emissions and extractions per person and per year, we first calculate the normalization score at endpoint level for each of the impact category, yielding results expressed in Disability Adjusted Life-Years (DALYs) for human health impacts, in PDF-m2-yr for impacts on ecosystems and in $ for resources. In order to put endpoint results on a comparable scale (in euros), we then apply the following societal weighting factors, from the stepwise method, derived from budget constraint: 74,000 Euro/DALY for human health, 0.14 Euro per PDF-m2-yr for ecosystem quality and 1 Euro per Euro for resources. Those values were applied on the normalization values of all the impact category indicators characterized at the endpoint level with the four LCIA methodologies, meaning that the result corresponds to the total monetarized impact in the world per person and per year predicted by each of the methodologies The IMPACT World+ methodology gives results relatively similar to the existing methodologies in most impact categories, while offering results for new categories such as water and oceanic acidification and differentiating in an additive way the short and long term of global warming. In the impact categories for which the results differ, it was possible to explain the difference of behaviour between the different methodologies and to relate them to different modelling assumptions, and to identify some strength and weaknesses of the different methodologies. Once results are calculated at endpoint, the proposed approach only requires two additional monetarized factors for human health and ecosystems to bring all results on a common scale. These can then be used as inputs to cost-benefit analysis and put in perspective the monetarized life cycle damages with revenues per person and year, using the budget constraint method.

MO219
Economic valuation of the hydrotreated vegetable oil in Spain using environmental externalities
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Substitution of biofuels by fossil fuels potentially involves important benefits to society, especially in the reduction of emissions of greenhouse gases. However, the use of biofuels entails emission of other pollutants in their entire life cycle, which must also be considered when assessing comprehensively the environmental consequences. All of these burdens impose risks on human beings, ecosystems and materials, producing damages that are *external* in the sense that they are not taken into account by the person or institution causing the effects. Quantifying these implications in a comparable manner would help to assess the global environmental performance of biofuels. When environmental impacts are expressed in terms of loss of welfare, using a monetary unit, *external costs* are obtained that can be easily used in a cost-benefit analysis or be internalized through the appropriate environmental policy instruments. The objective of this study was to assess, from an economic perspective, the environmental consequences of a biofuel obtained by means of oil hydrotreatment (HVO, Hydrotreated Vegetable Oil) in Spanish refineries. Results were compared to two counterparts: a fossil diesel < 10 ppm and another biofuel obtained in transesterification units (FAME, Fatty Acid Methyl Ester) in terms of the external costs produced by the changes in air quality. The evaluation concluded that FAME and HVO would have a worse environmental performance when compared to diesel due to the increased emissions of nitrogen oxides and ammonium. They cannot be offset by emission reductions that other pollutants present, such as sulphur dioxides and greenhouse gases. Nevertheless, results are highly dependent on the approach used to quantify the external costs of carbon dioxide emissions. Using carbon dioxide abatement costs instead of damage costs resulted in a better environmental performance of HVO compared to diesel in a 2020 scenario.

MO220
Economic valuation of nutrients removal from wastewater
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Wastewater treatment has important associated environmental benefits. However, in most cases these environmental benefits are not quantified because they have no market value. In spite of this, the monetary valuation of these externalities is necessary to justify the economic feasibility of wastewater treatment projects. Based on a cost production perspective, a valuation methodology has been developed for pollutants with no market value but substantial environmental effects. In this study, the economic value of removing nutrients from wastewater was estimated as a proxy to quantify the environmental benefits of reducing eutrophication impacts. Wastewater treatment can be considered as a production process in which a desirable output (treated water) is obtained together with a series of pollutants (nitrogen –N and phosphorus -P). Nutrientes removed from wastewater are considered undesirable outputs because if they were dumped in an uncontrolled manner they would cause a negative impact on the environment. The function distance and shadow prices for N and P were estimated for a sample of 16 wastewater treatment plants (WWTPs) located in Catalonia (Spain). The mean value of the N and P shadow price are -7.076 and -28.873 €/Kg– meaning that for every kg of N and P that is not dumped into the environment, the damage prevented, or the environmental benefit generated equals €7.076 and €28.873 respectively for N and P. By considering the volume of N and P removal in the treatment process (Kg/year) and the shadow prices for both pollutants (€/Kg) we can calculate the overall environmental benefit resulting from avoiding eutrophication in the 16 water bodies that receive the effluent from the sample of WWTPs evaluated. It has been estimated that this value is 15,893,923 €/year. The results are very useful to policy makers and stakeholders to justify the investment in wastewater treatment systems in areas where it is not required by law or to implement processess allowing higher pollutants removal efficiency.

MO221
Environmental Life Cycle Costing Applied to the Urban Water Cycle
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The continuous trend towards increasing urbanization brings to the fore the issue of urban sustainability. The AQUAENVEC project (LIFE10 ENV/ES/520) aims at providing decision-making tools to optimize eco-efficiency in the urban water cycle (UWC), through the environmental and economic life-cycle approach, Life Cycle Assessment (LCA) and Life Cycle Costing (LCC). LCC is a methodology that looks at the complete life span of a product, process, or activity and analysis the entire life-cycle cost. Within the scope of this study, firstly conventional LCC is undertaken to take into account internal costs. Several cost categories are estimated at the unit process level. This approach is chosen when the estimation of costs at the unit process level is straightforward or when the real aggregate values of a specific cost category are unknown. Costs that cannot be estimated at the unit process level are based on aggregate cost information and are broken down to the process level when appropriate. Further, accounting data is used to contrast the estimated costs at unit process level with real aggregate costs. Secondly, environmental LCC, which includes external costs, is carried out. Environmental impacts are firstly identified by LCA and then monetized. The environmental LCC is focused on the monetization of different LCA impact categories. Two small-medium cities located in the Mediterranean and Atlantic regions of Spain were selected as case studies for application of the aforementioned methodology.The system under study is the urban water cycle. It consists of four stages: (1) water extraction and drinking water treatment, (2) transport and distribution (3) sewer and (4) wastewater treatment. For each of these stages, conventional and environmental LCC is carried out comprising first costs, costs of operation, maintenance and major reparairs, demolition and final disposal. The applied LCC methodology allows to combine different data sets that are available at unit process and aggregate level. An advantage of this approach is that the cost assessment is very detailed while consistency of estimated costs with information on real costs is achieved. This study provides a methodological approach on how external costs can be monetized and incorporated in environmental LCC. The application of this methodology to such a complex system as the UWC helps to identify possible difficulties when applying such approach in practise and to propose solutions to overcome them.

MO222
Social indicators implemented in the sustainability evaluation for decision-making processes
P. Saling, A. Grosse-Sommer, BASF SE; J. Schoeneboom, BASF SE / Sustainability Evaluation
Processes and product development along the whole supply chain is a very important topic for introducing life cycle thinking and sustainability into business and customer relations. The use of Eco-Efficiency Analysis as well as

SEEBALANCE® or AgBalance™ analysis are key elements for doing so. Social impacts can be determined on the basis of stakeholder perceptions, public discussions, after initial hotspot analysis or by other processes. Contrary to the mere listing of indicators, indicator systems have a structuring framework which increases clarity. A classification of the indicators according to affected groups of persons appears suitable for this. Meaningful information can be integrated in different ways into indicator classifications and enables practitioners in different ways to evaluate and quantify impacts on social aspects. The indicators can be allocated in a meaningful way and the communication of the assessment results is clear and easily understood. In the development of SEEBALANCE and AgBalance, the following groups were identified as typical stakeholders that may be affected by social effects of production, use and disposal of products: Employees, Business partners, End customers and users as well as the neighbourhood and society at large. By adjustment to the sustainability topic, the groups Future generations and the International community Weaknesses and potentials driving social impacts can easily be identified and described in these main categories. Results are depicted in individual graphs of the respective category and initially do not contain any impact categories or weightings. The individual indicators per stakeholder are subsequently aggregated via the weighting factors to form the overall value for the stakeholder. This method will also be used for all other stakeholders of the social impact axis. These stakeholder issues are aggregated to another for the socio-efficiency with the weighting factors. The logic follows the Eco-Efficiency methodology but allows furthermore flexibility in the assessment of certain scenarios. It will be shown, how decision-making processes can be supported efficiently by using the sustainability Evaluation toolbox. Specific case studies focusing on social indicators and the data generation of important inputs will illustrate how decision-makers in the supply chain as well as in downstream applications can use these different methodological approaches.

Modelling techniques for future-oriented LCA and forecasting scenarios (P)

MO224
Environmental assessment of dynamic processes – considering time dependency in Life Cycle Assessment
E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; L. Tiruta-Barna, Université de Toulouse / INSA UPS INP LISBP; Y. Pigne, Normandy University / LITIS; N. Schiopu, Centre Scientifique et Technique du Batiment; A. Marvuglia, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTEResource Centre for Environmental Technologies CRTE
The introduction of time dependency in LCA has been dramatically underestimated and underexplored so far. In conventional LCA, human driven systems are typically considered to run in steady state conditions, neglecting time lags and stocks of goods and products. At best, the current practice considers different scenarios (related to time horizons) where relevant lifecycle inventory (LCI) parameters (e.g. related to the production functions like electricity production) are changed according to possible technological, market and regulatory evolutions. Following the same line of reasoning, the life cycle impact assessment models have limited coverage and consideration of dynamic features (related e.g. to pollutant fate, exposure and effect parameters). DyPLCA is a project jointly funded by the French and Luxembourgish research agencies (respectively ANR and FNR). The main objective of the project is to develop a comprehensive and operational approach for the proper consideration of time dependency in LCA, with strong emphasis on the development of an integrated modeling solution for both the LCI (at foreground and background levels) and the life cycle impact assessment (LCIA) phases. Results at the end of the project will be a methodology, models and computational tools for true dynamic LCA, well beyond the current practice based on forecasted scenarios and other recent research attempts, in a form readily usable by LCA practitioners. The modeling framework will be tested and applied to three relevant test bed LCA applications: 1) bio-technologies; 2) buildings and construction; 3) traffic noise. These systems were selected because of their contribution to the overall environmental impacts generated by human driven economies as well as because of the pertinence of the temporal scale in the assessment. DyPLCA will provide new scientific knowledge, clearly beyond the current state of the art of the science of LCA, focusing in particular on 1) the deepening and broadening the scope and modeling of LCAs in an rather unique way, through the combination of temporal characterization techniques and LCA and the harmonization of micro-process level inventories (i.e. ecoinvent v3 datasets) with time behaviors of large scale systems; and 2) full implementation of these modeling and investigation approaches on three practical application situations of broad societal interest.

MO225
Scenario analysis in Life Cycle Assessment of future hydrogen production through High Temperature Electrolysis based on Solid Oxide Electrolyser Cells
T.M. Bachmann, EIFER / Urban Systems
When analysing the environmental performance by means of Life Cycle Assessment (LCA), the functional unit regularly used for energy supply systems is the annual amount of useful energy provided. This disregards, however, the timing of energy supply as well as differences in terms of system deterioration due to varying numbers of ramp up and ramp down processes. In the EU project RelHY, these aspects have been taken into account in an LCA of future hydrogen (H_2) production by means of High Temperature Electrolysis (HTE) based on Solid Oxide Electrolyser Cells through the definition of different scenarios. These differed in terms of the origin of power and steam and the inclusion or not of back-up systems to cope with intermittency. The following five HTE-based hydrogen production scenarios were evaluated: operation with power and steam from a nuclear plant (NP), continuous and intermittent operation with wind power and water (WP vs. WPi), intermittent operation with natural gas or biogas reforming as back-up (WPi+NGR vs. WPi+BGR). Large scale natural gas reforming (NGR) was used as the reference scenario. LCI data for the manufacturing of the HTE stack and operation of the HTE plant were measured or calculated (e.g. electricity consumption with help of Aspen+) by the developers of the HTE. Data for generic upstream processes such as provision of the materials, energy and auxiliary and operating materials were taken from the ecoinvent data base version 2.1. Life Cycle Impact Assessment (LCIA) largely follows the ReCiPe 2008 hierarchist framework. The overall results for H_2 production show that all HTE scenarios studied lead to less life-cycle CO_2-equivalent emissions than NGR. However, only the wind powered HTE scenarios without back-up use less energy than NGR. The other impacts and flows show different patterns. Impacts are largely due to power supply. Even though having a limited contribution overall, stack manufacturing has the strongest impact during construction of the HTE plant. Its importance is even higher in case of intermittent power supply, requiring an assumed replacement in the scenarios with back-up (WPi+NGR and WPi+BGR) and two stacks to be operated in parallel to yield the same output in the case of the WPi scenario. Thus, improving the efficiency of the operation is the most promising pathway for most of the studied impacts. The study has been published as Patyk et al. (2013) Int J of Hydrogen Energy 38(10) 3865-3880.

MO226
Avoided life cycle impacts as a consequence to wind generation: Evidence that zonal congestion matters from Ontario (Canada) temporal data.
B. Amor, Universite de Sherbrooke / Civil Eng; M. Pellat, Stanford University; P. Pineau, HEC Montreal
Congestion, and therefore transmission capacity, is systematically neglected in consequential LCA when it comes to estimate avoided emissions due to a decrease of electricity production and vice versa. This simplification could be explained by the requested effort to integrate such mechanism into different models in consequential LCA, beside other market information's. To the best of the author's knowledge, this study is the first attempt towards quantifying the impacts of large-scale wind power (selected case study) in reducing environmental impacts from the power system, while taking into consideration internal congestion effects. The Ontario deregulated electricity market, over 6 years of market operations (2006-2011), is the selected case study to reach this objective. Using hourly data from Ontario (Canada) over the period 2006-2011, we establish that the impact of wind output on marginal emissions greatly differs depending on the observed congestion level within the system. Hourly data, without accounting for congestion, lead to misleading results that can overestimate the impact of wind output. As an example, result shows that avoided GHG emissions due to wind can be estimated to 331.93 tonnes per megawatt-hour (t/MWh) using all data (without taking into account congestion), while for uncongested and congested hours, avoided emissions are respectively estimated to 283.49 and 393.68 t/MWh. This work's main contribution is thus to underscore the importance of congestion in assessing impacts of wind. We also contribute to the literature in the approach we take to create clusters of data with respect to the congestion status and geographic coverage (congested zones of the grid). The presented results will help in bringing new thoughts into the advancement of the dynamics inclusion in LCIs with highlight on weak aspects of existing approaches. Keywords: Dynamics, Regionalisation, Transmission capacity; Cluster

MO227
A life cycle based environmental analysis of the resource wood: Toward future sustainable use patterns
F. Suter; B. Steubing, ETH Zurich; C. Mutel, S. Hellweg, ETH Zurich / Institute of Environmental Engineering
Wood is one of the most significant renewable raw materials and additionally a potential sink for carbon dioxide. Its versatility makes wood widely applicable for material and energetic purposes. Facing various future challenges, like a proceeding resource scarcity and a rapidly changing climate, it is of high importance to find smart solutions for using the diversity of the resource wood more sustainable and wisely in the future. This work aims to provide a life cycle based environmental analysis of the resource wood in Switzerland. Within the Swiss National Research Project 'Resource wood' (NRP 66) our approach should help to find strategies

enhancing the future efficiency and variety of wood use in Switzerland. For the environmental analysis of the current and future state of the wood value chain, the two methods of material flow analysis (MFA) and life cycle assessment (LCA) are combined. Hereby substitution, cascading and international trade of wood commodities are of special interest. As the main tool for the analysis and assessment we develop a mathematical model, which includes MFA and LCA functionalities. In a first step we model the material flows of the wood value chain. Thereby the demand for end-products drives the model. Through an intermediate products stage this also controls directly the total amount of required raw and recycling materials. In the model process outputs are split into main product(s) and material residues, each expressed as quantitative share of the total input. Process inputs are more flexible but restricted and controlled by material quality constraints. For this purpose raw and recycled wood are sorted into different quality categories, based on the variety of processing options for the respective material. In a second step LCA-functionality is implemented into the model. This combination of MFA and LCA model allows us to conduct scenario runs and environmental optimizations to answer the overall project question: What is the environmental performance of wood as a resource for materials and energy and how can it be used in the most resource efficient way? The results of this project should contribute to a sound and critical discussion about a desirable as well as possible use of the resource wood in the future. Ideally this provides strategies towards more sustainable use patterns of wood in Switzerland and worldwide. The poster will present the methodology of this work as well as preliminary results.

MO228

Allocation problem during the life cycle assessment of the process of coal gasification

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In a paper, issues related to the assessment of environmental burdens from multi-output processes were addressed. Assessment of environmental burdens has been performed using the Life Cycle Assessment (LCA). In case of multi-output technologies that have many functions, the question arises how to compare the environmental burdens of such technologies to the environmental burdens of competitive (reference) technologies? Comparative analysis of environmental burdens from the multi-output technology and a competitive reference technology requires so called “allocation”. That means that all the environmental burdens from the multi-output technology related to consumed resources and materials as well as emissions of gases and particulates to the air, solid wastes and wastewater throughout the production chain (the “life cycle”) should be allocated and assigned to individual products. In the paper the life cycle assessment of an exemplary object of the research were performed – the technological chain from cradle to gate of the process of joint production of methanol and power in the process of coal gasification. Two methods were used: system expansion (consequential life cycle assessment, CLCA) and the allocation proportional to the coefficients based on the existing relations between the products (attributional life cycle assessment, ALCA). In the study the question was posed: How large is the impact of the allocation on the results of environmental assessment? How may I chose the allocation method in the assessment of environmental burdens from multi-output technologies? Which technologies should be included when expanding a system? **Acknowledgements**
The paper has been prepared in the frames of the grant: "The methodology for allocation of environmental burdens in the life cycle assessment (LCA)", funded by the National Science Centre

MO229

Modeling of a glass mineral wool process in view of Life Cycle Analysis

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In line with the growing concern about the environmental impact of materials in the building sector, Knauf Insulation, a glass wool producer, is performing environmental impact assessment of its products through LCA. Knauf Insulation has several glass wool factories in Europe that produce various products, and for a specific market, the same product can be produced in several factories. As the plants that produce glass wool work with similar pathways, a generic model for LCA usable for every plant and every glass wool product has been designed. Moreover, combination of different factories is also possible. The general principle of glass wool production is the following: the raw materials, sand, limestone, soda ash, borax, sodium carbonate, as well as recycled off-cuts from the production process, are weighed and mixed. Knauf Insulation also uses a large amount of recycled glass (cullet). The mix is sent to a furnace at high temperature (1350°C). The melted material is then fiberized and the binder is added, a process called forming. Knauf Insulation uses a special binder with ECOSE Technology, a new and formaldehyde-free binder. The wool fibers are collected, by suction, on a conveyor belt, and the mattress then goes through the curing oven. For some product a facing is added. Finally the product is compressed and packed. The model, implemented in GaBi 6, is made as generic as possible by including, for each step, all the raw materials that can be used in one of the factories as well as all the energy sources. Parameters allow to define the amount of each raw material consumed, therefore the model can be adapted to any factory simply by setting these parameters accordingly. This also simplifies the data collection, since the template is the same for all the factories, it can be supported by data collection tools already existing. A part of the model is dedicated to weighting between factories, so a combination of factories can also be studied. The model can also be adapted to almost all Knauf Insulation products by using parameters where necessary: for example, several products have different binder contents, so a parameter defines the amount of binder. As some materials can be recycled between several parts of the process, special attention has been paid to recycling loops inside the model. The model is flexible enough to be used for Environmental Product Declaration (EPD) as well as for Eco-Design purposes.

MO230

Explorative LCA of the application of the CO2 capture to the cement production in Spain in 2030

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Cement production is one of the main sources of CO_2 emissions in Spain. Making cement requires great amounts of energy in form of heat since fossil fuels are burnt in the kiln. However, up to 60% of the CO_2 emissions come from limestone calcination with very difficult emission reduction strategies. For that reason, several solutions have been implemented in recent years in the form of Best Available Techniques as well as material and fossil fuels substitution scenarios. CO_2 capture is the most recent technology taken into consideration. In this work, it has been tested the implementation of post-combustion CO_2 capture technology using amines as absorbent in the Spanish cement production. It has been assumed that this technology will be available at commercial scale in 2030. The work discusses the pros and cons of implementing CO_2 capture technologies in cement production plants from an environmental viewpoint using Life Cycle Assessment. On the basis of the International Reference Life Cycle Data System (ILCD) 2011 impact assessment method, results show improvements in several impact categories such as climate change, ozone depletion and resource depletion. However, other categories such as photochemical ozone formation, acidification, eutrophication, human toxicity, ionising radiation, ecotoxicity, particulate matter, and land use show worse results. Moreover, the work shows the decisive contribution of the cogeneration plant required to produce process heat. More research is needed concerning the energy penalty linked to the post-combustion CO_2 capture. It is strongly recommended looking for synergies between cement facilities and power plants in order to optimise the heat consumption as well as exploring natural gas or biomass CHP plants.

MO231

Future-oriented LCA of high performance Flame Retardants

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Electrical and electronic (EE) applications are the largest market for flame retardants (FR) in plastics globally. The need for flame retardancy is increasing due to electronics miniaturization and higher temperatures in both processing and use. Existing FR based on halogenated compounds are known to pose severe environmental problems. The EU´s project PHOENIX (http://www.phoenix-eu-project.eu/) aims for the development of non-halogenated flame retardants based on nanostructural materials and biogenic resources. The research covers the entire spectrum from developing materials to their industrial application. Results will be used for decision making regarding long term investment and equipment, providing a significant advantage to participating SMEs but also society demanding environmentally friendly FR materials. Life Cycle Assessment is a generic part of the project research for a comprehensive evaluation of environmental characteristics. Overarching task is to predict how the environmental impacts of production and consumption systems related to FR will change in the future, taking into account the novel materials but also possibly changing environment and lifestyle. Two LCI modelling principles will be applied: • The attributional life cycle model will be applied for assessment of current halogen flame retardants. It is based on literature review of the actual material properties, processing knowledge and technical specification, and will be used as part of a BAU scenario for comparison of materials. • The consequential life cycle model will be applied for the new halogen-free FR. It covers modelling of the generic supply-chain as it is expected in consequence of the technical characteristics of the proposed FR additives, but also the expected market shares and use patterns. LCA is embedded in the work package concerning ecological and economic evaluation where information as to toxicological as well as economic performance of future materials is generated. The poster will present the conceptual outline of assessment as well as first results of the modelling or process chains,

including procedures for the comprehensive assessment of uncertainties which are growing due to the increasing fields of knowledge which are taken into account in consequentional modelling.

MO232

The use of Life Cycle Assessment in the development of nanofunctionalized membranes for virus and micropollutants removal

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Traditional water/wastewater treatment (WWT) systems consist of physical–chemical and chemical processes (e.g. coagulation–flocculation, settling, chemical disinfection, etc.) which are often energy - and resources - intensive processes. In the last decades, membranes processes have been used increasingly in the waste water industry as an attractive alternative to traditional processes. Nowadays, membrane processes are used for removing micropollutants and viruses from water streams. However, many efforts are needed to improve the membrane with respect to flux selectivity, and long-term performance in order to achieve to a less cost treatment for the production of drinking water . Within the EU-funded NANOPUR project (Development of functionalized nanostructured polymeric membranes and related manufacturing processes for water purification; EU Grant No. 280595), promising bottom-up technologies are applied to develop intensified water treatment concepts based on nanostructured and nanofunctionalized of microfiltration and ultrafiltration membranes. In order to evaluate potential impact posed by these new nanofunctionalized membrane, a Life Cycle Assessment (LCA) study was performed. Raw materials and auxiliaries processed in membrane manufacturing and its activation with atmospheric plasma process will be presented to provide a first inventory table and to assess the contribution of different substance and phases on the total environmental load. This work will also present a review of LCA studies dealing with WWT, in order to emphasize the variability in terms of functional unit, system boundary, and data used. The quantification of possible release of added functionalities from new membranes during the pilot test trial runs will be also discussed in this LCA study, as well as the energy and materials involved in membrane operation (chemical cleaning, back-washing, etc.). The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 280595.

MO233

MFA + LCA applied to industrial parks

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To bring sustainable resource use into practice it is important to have concrete measurements. Material flow analysis (MFA) has become a useful tool for industrial ecology to analyze the metabolism of social systems, such as countries, regions and cities. This contribution proposes to use the LCA indicators obtained with the metabolism description considering their production process. MFA+LCA wants to analyze the efficiency and the materialization ranks of industrial areas, as well as the main impacts due to the flows. The industrial park metabolism analysis will use the LCA methodology to estimate the environmental impacts of a large list of products produced/consumed in the industrial park. The methodology is applied to a case study of a new industrial area located in Sao Carlos(Sao Paulo, Brazil) and compared with a service park in Catalonia (Europe). The methodology proposed has been adapted from previous works where industrial parks were analysed with MFA (Sendra et al., 2007), and the urban metabolism studied combining MFA, LCA and Extended Input Output analysis (Dias et al., 2013) for urban areas (Aveiro). To get a broader picture of the environmental impacts of the industrial park was first done an analysis of its characteristics and metabolism. The MFA will be completed with the LCA process. Therefore in the second step it was done a LCA for the inputs of the industrial park, considering as many input products as possible. The functional unity was defined to be the production/consumption of one worker per day and for most products the production/consumption was assigned in kg per worker per day (kg/work/day). The definition of the products to be considered was based on their relevance in terms of mass and economic share within their correspondent industry.National data of industry sales to the domestic market by product type were used. As these data on industry sales and imports are available only at the national scale, a downscaling approach was applied in order to obtain data for the Sao Carlos area. An specific analysis was done in order to avoid double counting of impacts. Sendra, C, Gabarrell, X, Vicent, T . Material flow analysis adapted to an industrial area. Journal of Cleaner Production 15 (2007) Dias, A, Lemos, D, Gabarrell,X, Arroja, L. Environmentally extended input-output analysis on a city scales application to Aveiro (Portugal) (Submitted, 2013)

MO234

Life cycle assessment linked with process models for the development of water reclamation processes

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A study of combining full-scale, pilot scale test data with theoretical models of a multi-stage wastewater reuse plant is being carried out with the aim to find environmentally optimal modes of process operation. Different scenarios pertaining to the treatment process as well as to the surrounding infrastructure are modelled and then environmentally assessed based on data mainly from full-scale plants but also pilot tests. The present study gives a practical demonstration how modelling and LCA can be utilised together as a tool for analyses and planning of water reuse schemes, taking into account process parameters as well as parameters, which describe the surrounding infrastructure. Based on the experimental results, equipment design data, model calculations and with Xylem expertise on equipment, models of full-scale plants are created in Matlab. These models are used to deliver input data to LCA models (in GaBi-software). The LCA models are then supplemented with modules describing the surrounding infrastructure. The main variables when modelling the treatment system in Matlab are technology design and plant size. The result of the study an example how we use modelling at product and system level in combination with LCA to design environmentally “best-available” technologies for water reclamation and assess these technologies against the benefits of reduced use of water as a resource.

MO235

Modular - LCA - Approach – Find the best combination using the example of components of small hydro power plant

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In Germany, due to a challenging climate and energy policy, renewable energies need to be extended. This also covers hydropower. However, potential for sites for large-scale hydro power plants is almost depleted in Germany. In contrast, many sites for small-scale hydro power plants still exist. However, specifically small plants are discussed in a very controversial manner. On one hand, huge progress has been made regarding components for small hydro power, where performance is at a high level of efficiency. On the other hand, it is suspected that part of these efficiency gains are jeopardized by the small scale of plants as well as by the necessity of having structure components for ensuring ecological compatibleness. There are different technologies established which lead to a very high variability of possible set-ups of small water power at a specific site. Present life cycle assessment (LCA) does neither account for this high variability of small-scale water power nor for current technological performance and possible learning effects. To tackle these problems, a modular LCA-approach is developed to determine the environmental impacts for energy production as well as the ecological compatibility of small hydro power plants. At first, a technology-matrix is developed where all site-specific components will be defined. Additionally, the technologies will be completed with learning curves to evaluate further developments for components. Based on this information, modules for process chains of full installations of small hydro power are defined. For each module, data sets will be compiled basing upon methodological principles that have been developed for modular LCA of bioenergy (www.bioenergiedat.de).The environmental impacts of each concept are evaluated with a focus on materials, energy return of invest and emissions (carbon dioxide and methane). Further, for ecological compensating measures, soil has to be transformed, which in turn might influence stored CO_2. Finally, methane emissions due to water storage are investigated. With this modular LCA-approach, the environmental impacts of different technological concepts can be determined for each site specifically now and in the future. From this findings, suggestions for choosing components can be derived. Additionally energetic efficiency as well as the cumulative environmental impacts can be assessed in order to investigate implementation of small hydro power plants on specific sites.

MO236

Nanofibers and Pyrolysis - From the laboratory scale towards a theoretical industrial scale: error propagation and uncertainty analysis in scale-up process for prospective environmental assessments

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Life cycle assessment (LCA) and other similar methods can broaden and deepen the understanding of technological processes and define their environmental, economic

and social hot-spots. These methods are increasingly used for mid-term decision-making and long-term policy planning by governmental and non-governmental organizations as well as by companies in the last decade. The continuous progress of technology constrains scientist and process developers, analyzing and evaluating prospectively a given technology during its development phase. On the other hand, the flexibility of the design in the early development phase allows changing the technology regarding the possible environmental, economic and social impacts. A prospective assessments needs to model immature technologies by scaling-up from laboratory scale onto industrial scale, in order to understand the strength and weakness of the new material/technology on a product level. But this procedure can have a significant inherent uncertainty. This type of scaling-up is entirely theoretical and it can cause significant uncertainty in results. Therefore the proper evaluation of the probability of the results is also highly important. This study investigates the scale-up procedure of three different processes. The object of the first scale-up method is the electrospinning process for nanofiber manufacturing, and the two others deal with pyrolysis technology. An error-propagation is conducted from the laboratory experiments to the modeling of fictive industrial scale. This informs us how the observational errors of laboratory measurements affect the result of scale up. A sensitivity analysis of the three models shows how the changes of process parameters affect the results. This analysis also investigates the calculation errors and uncertainty of assumptions which are used in the LCA-model. Additionally a contribution analysis defines the hot-spots and important flows of the processes, and points out the highly important input-output and interim flows which have to be regarded during the scale-up procedure.

MO237

Life Cycle Assessment of flat plate and evacuated tubes solar water heaters in Lebanon

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Lebanon lacks a reliable, secure, and environmentally acceptable energy system. The country suffers from frequent power outages, high electricity bills, transmission and distribution losses, and high levels of greenhouse gas (GHG) emissions per kWh of power production. Thus, it becomes a fundamental challenge to satisfy the growing demand for energy in a manner that is both safe and environmentally acceptable. Therefore, the development of a sustainable energy system becomes a necessity. Because Lebanon lacks the use of fossil fuel sources, 98% of its energy is imported to meet the country's electricity needs that is mostly satisfied with thermal power plants. Hot water delivery in the residential sector is mostly done via electric boilers that expend a lot of electricity. To this end, increasing the penetration of solar hot water systems has the potential of reducing electricity use. Lebanon is endowed with climatic and topographic variation, allowing it to benefit from a variety of renewable energy sources, in particular solar energy. However, little research has been undertaken to assess whether or not the environmental performance of SWH is truly superior to using conventional water heating methods if impacts from the production, use and disposal of the SWH are thoroughly internalized. For this reason, a life cycle assessment (LCA) is performed. The objectives of this study are to i) evaluate the environmental impacts of the technologies used in the Lebanese Electricity Grid (LEG) and ii) determine and compare environmental impacts of two types of SWH installed in Lebanon (Flat Plate (FP) and Evacuated Tubes (ET)) using Life Cycle Assessment (LCA). The inventory analysis of the LCA is done using the SimaPro software with data collected from Lebanese SWH manufacturers and the potential impact assessment is performed using the IMPACT 2002+ methodology. For the LEG system, results indicate that damage is mostly caused by electricity production using a steam-turbine power plant followed by combined-cycle power plant. To improve this, natural gas can be used to operate the power plants instead of diesel oil. For the SWH systems, the flat plate is a better installation choice environmentally in regards to all the impact categories except for aquatic eutrophication. As for the components of SWH systems, the storage tank is contributing the most to impact categories for the FP, while the absorptive coating in ET has the highest contribution to impact categories.

Assessing the risk of environmental pollutants on amphibians and reptiles (P)

MO238

Influence of temperature on the lethal and sublethal toxicity of hydrophobically modified polyacrylic acid on tadpoles of Pelophylax perezi and Epidalea calamita

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Crosslinked polyacrylic acid derivatives have been used as promising materials for drug carrier/delivery systems, namely when dealing with poorly absorbed therapeutic drugs. Their efficiency in this type of applications is related with their stimulus-responsive nature, i.e., capacity to experience swelling/deswelling under varying environmental conditions, like for example pH and temperature. The fast advances in this industry are causing the introduction of such materials in the environment, specifically into the aquatic ecosystems, thus, representing a threat to ecosystems' resilience. Amphibians are excellent bioindicators to study the risk associated with the release of these materials into the aquatic environment, since they inhabit a variety of habitats associated with freshwater sources. The objective of this study was to evaluate the lethal and sublethal effects caused by the crosslinked hydrophobically modified polyacrylic acid-HM-PAA in tadpoles of two species of amphibians: *Pelophylax perezi* and *Epidalea calamita*. Tadpoles, from each species, at Gosner stage 25 were exposed, for 168 hours, to a range of six concentrations of HM-PAA plus a control (FETAX medium) at temperatures of 20°C and 25°C, according to standard FETAX protocols. The suspensions of HM-PAA were characterised for size, aggregation index, zeta potential, surface charge, pH, conductivity and dissolved oxygen. The following endpoints were assessed during or at the end of exposure: mortality, malformations, total body length (TBL), total body weight (TBW) and enzymatic biomarker levels (GST, LDH, AChE, and CAT) where measured. Results revealed that temperature did not interact with HM-PAA toxicity. HM-PAA significantly affected both TBL and TBW, in both amphibian species. The tested concentrations of 1.8 and 9.3 mg/L caused a significant decrease in TBL at 20º C for *E. calamita* tadpoles, when compared with the control. Such significant differences were not observed in *P. perezi*. Regarding TBW, *E. calamita* presented significantly differences at 25º C for the concentration of 2.8 mg/L. Differences were also observed in *P. perezi* at the same temperature but in the concentration of 4.1mg/L. As *P. perezi* showed no significant mortality rates (≤ 10% at the two temperatures), it is suggested to be more resistant to HM-PAA NM than *Epidalea calamita* where mortalities of 16% were registered at 20ºC. Results for biomarker levels are still being analyzed and will be presented in the poster.

MO239

COPPER-DRIVEN AVOIDANCE AND MORTALITY BY TEMPERATE AND TROPICAL TADPOLES

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Amphibians have experienced an accentuated population decline in the whole world due to many factors, such as climate change, fungal diseases, exotic species, and UV radiation. Anthropogenic contamination seems also to play a crucial role. Therefore, the present work aimed to assess the potential role of copper, as a worldwide and reference contaminant, on the exposed population immediate decline due to avoidance and mortality responses in tadpoles of three species of amphibians: a South American species, *Leptodactylus latrans, a North American species, Lithobates catesbeianus,* and an European species, *Pelophylax perezi*. A non-forced exposure system with a copper gradient along seven compartments through which organisms could freely move was used to assess the ability of tadpoles to detect and avoid copper contamination. All species were able to avoid copper at a concentration as low as 100 μg L^{-1}. At the lowest (sublethal) concentrations (up to 200 μg L^{-1}) avoidance played an exclusive role for the population decline, whereas at the highest concentrations (>450 μg L^{-1}) mortality was the response determining population decline. The median population immediate decline concentrations were 93, 106, and 180μg L^{-1} for *Le. latrans*, *Li. catesbeianus* and *P. perezi*, respectively. Contaminants might, therefore, act as environmental disturbers both by generating low quality habitats and by triggering avoidance of tadpoles, which could be an important response contributing to dispersion patterns, susceptibility to future stressors and decline of amphibian populations (together with mortality).

MO240

A database to assess the sensitivity of terrestrial amphibians and reptiles to chemicals compared to other taxonomic groups

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The new regulation for plant protection products (1107/2009) requires the risk to terrestrial amphibians and reptiles be assessed using all available information. Currently there are no appropriate validated acute and chronic toxicity test methods for these groups, furthermore additional vertebrate testing should not be required unless it is absolutely necessary. In an attempt to address this issue, the German agrochemical industry association (IVA) has initiated a project to collect relevant information for terrestrial amphibians and reptiles from the literature. The project will build on the data previously collated for the European Food Safety Authority (EFSA). Toxicity data for terrestrial amphibians, reptiles and other groups (worms, fish, birds and mammals) will be analysed to investigate relative sensitivity and any potential correlations amongst the groups. Other supporting information, which may help interpret any relationships, such as bioconcentration values, physico-chemical properties (water solubility, logPow etc.) as well as dermal adsorption studies conducted for human health assessments will also be compiled. This poster will summarise the database constructed and present the outlook for possible analyses.

Current Developments and Challenges on Sediment toxicology in Scientific and Regulatory Contexts (P)

MO241

Relevance of a risk based approach in the management of contaminated sediment: perspective and experiences from Europe

E. Bizzotto; S. Deacon, ENVIRON UK Ltd; F. Colombo; R.J. Wenning, ENVIRON International Corporation

In Europe, legislation for the protection of the aquatic environment typically requires the achievement of defined environmental quality standards in water. Recently, progress has been made towards the development of an EU regulatory framework for sediment risk assessment, including the development of similar standards for sediments. The definition of sediment benchmarks and, more generally, their use in the evaluation of contaminated sediment is challenging since the toxicity and bioavailability of contaminants can be strongly influenced by a variety of site-specific parameters and conditions. If remedial decisions are taken based on the outcome of a comparison with generic benchmarks this can have strong implications for the management of contaminated sites, in particular the costs of cleanup and the potential unintended consequences of unnecessary actions. The poster will discuss the relevance and significance of an EU-wide approach and the need for a European risk assessment framework that allows for flexible, realistic and site-specific risk assessment in the evaluation of contaminated sediment sites. Experiences and results from case studies will be presented.

MO242

Evaluation of sediment toxicity and risk assessment for benthic organisms: a case study

E. Bizzotto; P.C. Fuchsman, ENVIRON International Corp; F. Colombo,

Sediment quality benchmarks are intended to predict sediment toxicity but often are derived in a manner that does not necessarily reflect cause-effect, concentration-response relationships. Therefore, although generic benchmarks can be considered useful for screening purpose, care should be taken before using them to define need for remediation. Instead, as reported in several guidelines, the real need for remediation should be evaluated considering site-specific condition and risk assessment. We present and summarized the approach adopted to evaluate risks of sediment-associated DDx to benthic invertebrates, considering several lines of evidence including (1) toxicity studies and bioaccumulation test performed on field collected sediments, (2) evaluation of site specific ecological conditions, (3) review of toxicity testing and benthic invertebrate community assessments from major DDT-contaminated sites, and (4) extrapolation of aquatic toxicity data to sediment using the equilibrium partitioning approach. The results are discussed as evidences to support the decision making process for the management of a contaminated sediment case study.

MO243

Comparison of first tier risk assessments for plant protection products with sediment-spiked and water-spiked studies

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According to the current aquatic guidance document for the risk assessment of plant protection products (EFSA 2013), the impact of an active substance on sediment-dwelling organisms shall be assessed when accumulation of the substance is indicated or predicted by environmental fate studies. *Chironomus riparius* is used as a standard test species, and the current data requirements allow applying the active substance either to the water or the sediment phase of a water/sediment system. No effect concentrations (NOEC) from water-spiked studies should be compared to predicted environmental concentrations (PEC) in water bodies, while NOEC from sediment-spiked tests should be compared to PEC in sediments. We were interested, in how far the choice of the test system influences the first tier risk assessment for sediment dwelling organisms. We have chosen 15 active substances for which both, water-spiked studies and sediment-spiked studies with *Chironomus riparius* were available. A first tier risk assessment was performed for both exposure routes. NOEC from water-spiked studies were compared to worst case PEC in water bodies and NOEC from sediment-spiked studies were compared to worst case PEC in sediments. This allowed a direct comparison of toxicity exposure ratios (TER = NOEC/PEC) from water-spiked and sediment-spiked studies. TER derived from water-spiked studies were generally lower or comparable to TER from sediment-studies. The data suggest that first tier risk assessments for *Chironomus riparius* thus can be solely based on water-spiked studies. Sediment-spiked studies might be rather used for higher tier risk assessments, where more realistic exposure schemes are applied.

MO244

Ecotoxicological effects of contaminated sediments on fish: Are current risk assessment protocols sufficient?

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The water quality of the Rhine River improved substantially over the last decades, and today most fish species that were endemic before industrialisation are established again. Contaminated sediments, however, remain an important exposure pathway for the aquatic fauna. We tested sediment samples of a former gravel pit lake (quarry pond Karlskopf) connected to the Rhine River for potential ecotoxicologically adverse effects on fish using five bioassays: (1) acute cytotoxicity test (cytotoxic effects), (2) EROD induction assay (dioxin-like effects), (3) L-YES (estrogenic effects), (4) fish embryo toxicity test (teratogenic and embryotoxic effects), and (5) immobilization test with *Daphnia magna* neonates (acute toxicity). The test with *D. magna* was conducted using pore water, whereas the other biotests were performed using either organic extracts or native sediments. All samples induced dioxin-like, estrogenic, teratogenic, embryotoxic and cytotoxic effects, although no acute toxicity on *D. magna* was observed; indicating that the contamination was not readily bioavailable through pore water. Cytotoxicity was in accordance with previous studies on the Rhine and Danube River. In contrast, dioxin-like effects were higher than the ones described in studies on the Neckar, Danube and Rhine River. The L-YES revealed estrogenic potentials of the sediment extracts. Although sediment contact tests with *Danio rerio* embryos showed virtually no mortality, sublethal effects were evident in almost all samples. This indicates that smaller contaminant fractions partition into the waterphase and can impact benthic organisms. Results of the five biotests did not correlate, suggesting that they were caused by different groups of contaminants. Cluster analysis suggested that incidences of contamination in the pond increased with increasing distance to the main channel. The pore water test with *D. magna* represents the official German risk assessment for dredged freshwater sediments, yet results from four biotests indicate that risk assessment protocols would benefit from incorporating sublethal and chronic effects on vertebrates. At the same time the relation of biomarker response to general fish health needs to be further substantiated. Our results suggest adverse effects of sediments of the quarry pond Karlskopf on fish and suggest the need for revising risk assessment procedures.

MO245

Toxicity of sediment-bound triclosan, triclocarban, irgarol and cypermethrin to the freshwater ostracod Heterocypris incongruens (ISO 14371).

M. Casado-Martinez, Centre Ecotox; R. Bebon, C. Gachet-Aquillon, N. Homazava, Swiss Centre for Applied Ecotoxicology; E. Vermeirssen, Eawag / Dept of Environmental Toxicology; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology

The toxicity test using freshwater crustacean ostracods of the species *Heterocypris incongruens* has been commercialised since 2002 and an ISO standard has been published recently (ISO 14371-2012). Toxicity tests with field-collected sediments have shown that results of mortality and growth in ostracods compare well with those from other traditional sediment bioassays (*Hyalella azteca, Chironomus riparius*) while offering many advantages: it is the only sediment contact test commercially available; test organisms can be obtained from cysts so culturing is avoided; it requires a small quantity of sample and bench space; and it is easy to run and interpret. Despite the relatively long exposure duration (6 days), it is highly "doable" on a routine basis and thus suitable for screening large numbers of samples. We carried out tests to establish the sensitivity of *H. incongruens* to four organic micropollutants that are highly relevant for the sediment compartment due to their relatively high partitioning coefficients and widespread use: triclosan, triclocarban, irgarol, and cypermethrin. Chemicals were spiked into artificial sediments (ISO 2012-16191), aged, and then tested at 5 different concentrations. According to the lethal effect concentrations (LC50), the sensitivity of *H. incongruens* to the tested chemicals in decreasing order is cypermethrin > triclocarban > irgarol ~ triclosan and according to the sublethal effect concentrations (EC50) cypermethrin > irgarol ~ triclocarban > triclosan. Results obtained will be compared to effect concentrations for other standard test organisms and measured environmental concentrations. Results will be discussed in relation to the use of this test system for prospective and retrospective risk assessment of the sediment compartment.

MO246

Zoning and spatiotemporal evolution of PAHs, described combining sediment concentration, baseline toxicity, bioaccumulation in fish and TEC for fish

E. Rojo-Nieto, CactymarUniversity of Cadiz / Department of Environmental Technologies; J. Perales, CACYTMAR University of Cadiz / Department of Environmental Technologies

In this work, fate and effects of PAHs in the Bay of Algeciras, a semi-enclosed coastal zone subject to an intense industrialization, have been studied. This Bay has suffered a chronic anthropogenic pressure, due to urban (five urban areas with more than 250,000 inhabitants) industrial activities (petrochemical and metallurgical industry), and to the intense maritime traffic (Algeciras Harbor is ranked among the most important ports of the world). In previous studies occurrence and levels of PAHs in sediment were studied, and a zoning of the bay according to contamination level and source was proposed. Additionally, sources, transport and fate of these compounds in sediments were studied obtaining a spatiotemporal evolution. The aim of this work was to study if different parameters related to biota, as Bioaccumulation in feral flatfish (BSAF), Baseline Toxicity for marine organisms (described by the chemical activity) and Toxicity Equivalent Concentration for fish of PAHs found in sediments (TECs), could provide a similar zoning and spatiotemporal evolution that produced by sediment concentrations, to define PAHs pollution in the study area. The results obtained show that the study of the total concentration, the interstitial concentration and the chemical activity of PAHs, in marine sediments from chronically polluted environments (and the translation of them to other biota-related parameters, through well-defined factors), provides fairly accurate knowledge about the distribution and spatiotemporal evolution of the environmental risks associated to their presence. However, since in natural environments rarely the environmental compartments are in total equilibrium and since the biological effects and bioaccumulation are influenced by several factors such as the metabolization of the compounds under study, the analysis of biotic compartment can not be completely ignored, being this compartment essential to define, among others, site/species-specific BSAFs.

MO247

Heavy metals, trace elements and sediment geochemistry at four Mediterranean fish farms

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Fish farm wastes can accumulate on sediments below or near the fish cages. This organic material represents a potential risk of contamination to the wider environment, exhibiting a variety of biological, chemical and ecological effects. Changes in the redox regime, free sulphide and organic matter affect the behavior of various metals and element species in sediment. Sediments can scavenge some elements, thus acting as an adsorptive sink. However, sediments are not only a sink but also a possible delayed source of these contaminants into the water column due to desorption, remobilization processes, redox reactions and degradation of sorptive substances. Fish farming sites occasionally represent “hypoxic or anoxic islands” in the highly oligotrophic Mediterranean Sea which are likely to induce changes in metal and element behavior and their interactions with the local marine organisms. Trace element concentrations in sediment were investigated at four fish farms in the Eastern Mediterranean Sea. Fish farms effects were negligible beyond 25 – 50 m from the edge of the cages. Based on elemental distribution, sediments from the farms were separated into coarse oxidized and silty reduced ones. Fish feed is richer in P, Zn and Cd than reference and impacted stations. Comparison among impacted stations and the respective reference stations shows that, in anoxic sediments, all elements had higher concentrations at the impacted stations than at reference stations while in oxic sediments, many elemental concentrations were lower at impacted stations than at reference stations. The behavior of elements and therefore their distribution is affected by changes in sediment grain size, organic content and redox regime. Elements in sediments around fish farms can be clustered into five groups according to these environmental variables. In silty and anoxic sediments, element concentrations were higher than in coarse and oxic ones. Several approaches were used to assess potential sediment toxicity (enrichment factors, geoaccumulation indices, contamination factors) as well as to assess the potential danger to aquatic life (Sediment Quality Guidelines, SQG). Cu, Zn and Fe can cause from threshold to extreme effects on aquatic life in anoxic, fine-grained sediments and As can cause threshold effects in all types of sediment around fish farms. Other elements (Cr, Pb, Mn) can also cause unwanted effects when compounded with elevated background levels.

How can scientific advances support regulatory risk assessment for pesticides? (P)

MO248

Pesticide usage data for the application of the research to be utilized for IPM actions?

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To collect regularly the data of pesticide usage on target plants is rather new action in EU (1185/2009/EC). In addition to this, in the EU strategy the aim is to reduce risks of used pesticides to a minimum (2009/128/EC) via IPM (integrated pest management). However, in this study we were able to obtain pesticide usage data from Finnish crop production fields over 2002-2011 that covered about 10 % from all crop farming in Finland. The data was received from the Pro Agria Advisory Centres. The usage was compared for sales data in Finland, surveyed by Finnish Safety and Chemical Agency (TUKES). The potential environmental impacts for 64 pesticides (about 180 pesticides sold over the time scale) were calculated with the SETAC consensus LCIA (life cycle impact assessment) model of Usetox, customized to fit Finnish regional environmental conditions. PestLCI 2.0 was used to estimate the emissions assuming average Finnish field conditions. In results, The most of the impacts induced fungicide fluazinam (used on potato), herbicide aclonifen (e.g.peas,carrot,onion), fungicide prochloraz (cereals, oil seeds) and fungicide mancozeb (on potato), respectively. Even thought, herbicides were the most used pesticides, fungicides were the most hazardous ones from the pesticide groups. The usage data corresponds to the sales (R-value 0.955). Consequently, the results via this LCIA approach enables to make changes in environmental management; to change chemicals to more environmental safe ones or to give options to change methods in the agriculture towards to more environmental friendly way. In conclusion, this work can enable to advance IPM actions in the usage of pesticides in a farm level, and thus to provide tools to sustainable plant protection.

MO249

The use of sediment, soil or soil extracts in the Daphnia magna reproduction test following the OECD TG 211

S. Hoeger, Innovative Environmental Services IES Ltd / Environmental Toxicology; S. Streil, A. Peither, Harlan Laboratories Ltd

The *Daphnia magna* reproduction test following the OECD TG 211 represents a well established test during the risk assessment of chemicals, pharmaceuticals, biocides and agrochemicals. Due to special characteristics of the test substance and under certain circumstances such as adsorption of the test item to the sediment or to substances leaching from sediment it can be appropriate to include natural or artificial sediments such as sandy or earthy sediment or extracts of those in the test design. These modified test designs provide a more realistic exposure scenario for the investigation of the effect of test substances in the environment (higher tier tests). Neither the OECD TG 211 nor the OECD TG 23 (Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures) mention these modified test designs. As the inclusion of sediment or leaching substances changes the environment for the daphnids decisively, the test specifications and validity criteria for the standard test listed in the OECD TG 211 may not be met. Therefore, detailed pre-experiments are necessary to check for instance the reproduction (validity criterion > 60 juveniles/adult) and to develop a feeding regime which provides sufficient reproduction during the test. In addition, the behaviour of the test item during the test intervals has to be observed. Information of possible adsorption of the test substance to components of the sediment is important as the adsorption could influence the bioavailability of the test substance. From a practical point of view the method of separating the offspring from the water sediment system and the preparation of the sediment water system before the test medium renewal periods requires intense considerations and pre-experiments. In this presentation possible test designs will be introduced and the advantages and disadvantages will be discussed. The experiences during the development of theses different study designs will be summarized in a proposal to amend the OECD TG 23 and/or 211.

MO250

Statistics matter: data aggregation improves identification of community-level effects compared to a commonly used multivariate method

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The identification of the effects of toxicants on biological communities is hampered by the complexity and variability of communities. To overcome these challenges, the trait-based SPEAR approach has been developed. This approach is based on (i) identifying the vulnerable taxa using traits and (ii) aggregating these taxa into a group to reduce the between-replicate differences and scattered low- abundance distribution, both of which are typical for bio- logical communities. This approach allows for reduction of the noise and determination of the effects of toxicants at low concentrations in both field and mesocosm studies. However, there is a need to quantitatively investigate its potential for mesocosm data evaluations and application in the ecological risk assessment of toxicants. In the present study, we analysed how the aggregation of the sensitive taxa can facilitate the identification of the effects. We used empirical data from a long-term mesocosm experiment with stream invertebrates and an insecticide as well as a series of simulated datasets characterised by different degrees of data matrix saturation (corresponding to different sampling efforts), numbers of replicates, and between-replicate differences. The analyses of both the empirical and simulated data sets revealed that the taxa aggregation approach allows for the detection of effects at a lower saturation of the data matrices, smaller number of replicates, and higher between-replicate differences when compared to the multivariate statistical method redundancy analysis. These improvements lead to a higher sensitivity of the analysed systems, as long-term effects were detected at lower concentrations (up to 1,000 times). These out- comes suggest that methods based on taxa aggregation have a strong potential for use in mesocosm data evaluations because mesocosm studies are usually poorly replicated, have high between-replicate variability, and cannot be exhaustively sampled due to technical and financial constraints. For details see: Beketov M.A., Kattwinkel M., Liess M., 2013. Statistics matter: Data aggregation improves identification of community-level effects compared to a commonly used multivariate method. Ecotoxicology, 22: 1516-1525.

MO251

Laboratory aquatic invertebrate single-species screening studies

R. Zivtins, Cambridge Environmental Assessments; H. Walton, ADAS UK Ltd; F. Joyce, Cambridge Environmental Assessments

The conduct of acute aquatic toxicity testing in the laboratory provides an efficient means of predicting the effects chemicals may have in the environment, particularly for short-term studies. As such, these tests can aid regulatory risk assessments by providing confirmatory or additional data to support regulatory submissions. We will present the results of recent work carried out by Cambridge Environmental Assessments (CEA) in this area with a number of non-standard invertebrate species. To test the effects of a chemical substance, a range of freshwater invertebrate taxa were used in a screening study. Planaridae, Lymnaeidae, Baetidae, Chaoboridae, Cyclopidae, Chironomidae, *Culex* sp., *Crangonyx* sp., *Asellus aquaticus*, Coenagrionidae were exposed for 48 or 96 hours to series of exposure concentrations. Organisms were other tested one per vessel or 5 per vessel, depending on their life history characteristics. Daily (24hr) observations for a number of parameters were made at 24-hour intervals however the key endpoints were immobility and mortality. We present the methods used for collecting, acclimating and culturing these freshwater invertebrates and also make recommendations for standardising exposure durations and relevant effect endpoints for each taxa.

MO252

Dynamics and risk assessment of pesticides detected in surface water of the Alqueva reservoir (Guadiana basin; south of Portugal)

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Freshwater reservoirs located in intensive agricultural areas are more vulnerable to chemical "stressors", such as pesticides. Hence, the characterization of these water bodies for prevalent pesticides is extremely important, once most of these compounds are used in an indiscriminate way by farmers and induce toxic effects in species of aquatic ecosystems and benthic communities. The purpose of this study was to evaluate the dynamics of the principal pesticides detected in the Alqueva reservoir and evaluate their potential impact on the aquatic organisms of this ecosystem. For this purpose, the the occurrence of 25 pesticides and some of their degradation products was determined in surface waters from Alqueva. The target pesticides, which belonged to the classes of phenylureas, triazines, chloroacetanilides and organophosphorous, were analysed by isotope dilution on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. The aquatic risk assessment, which was based on the risk quotient method (RQ=MEC/PNEC), considered three trophic levels: algae, aquatic invertebrates and fish. The pesticides more frequently detected were bentazone, terbuthylazine, metolachlor, MCPA, and 2,4-D. The areas (sampling stations) most polluted by pesticides were Sra. Ajuda, Lucefecit and Álamos, located in the northern and in the middle of the reservoir, respectively. The aquatic risk assessment revealed that from the various compounds analysed terbuthylazine, chlorfenvinphos and diazinon presented non-acceptable risk when maximum concentrations were used as measured environmental concentrations (MEC). The location that had more samples with risk quotients higher than 1 (high risk) was Ajuda followed by Lucefécit. The use of risk assessment allowed concluding that despite pesticides concentrations in the water column fulfil the European environmental quality standards, some of the compounds show high ecotoxicological risk for aquatic organisms of the Alqueva ecosystem. The results thereby demonstrate that for an efficient risk management process the regulatory authorities of each country must consider an integrative chemical and ecotoxicological approach.

MO253

Breeding success in birds exposed to treated seed: methods & results

A. Lawrence, Cambridge Environmental Assessments; J. Crocker, Food and Environment Research Agency

We will provide an update on UK Chemicals Regulation Directorate funded project PS2373 “Development of reproductive risk assessment methods for birds potentially exposed to treated seed”. Avian long term risk assessments are designed to ensure that, following a pesticide application, reproductive effects are unlikely and that there will be no long term repercussions for abundance and diversity (EFSA, 2009; Regulation EC 1107/2009). Conventional avian reproductive risk assessments for treated seeds have a relatively high ‘failure’ rate (indication of potential risk), but the inherent conservatism would suggest a proportion of these failures are ‘false positives’. The conservatism comes from an assumption of coincident breeding and drilling activity and a ‘one field’ approach to the risk assessment, in addition to the use of worst case toxicity endpoints, which may relate to a specific breeding phase which may not be exposed in reality. We modelled the effects of pesticide seed dressings on the reproductive success of birds of arable land. We investigate the effect of the timing of seed sowing of 3 spring crops on the breeding success of 4 arable bird species (rook, linnet, skylark, yellowhammer). We ran two types of model, a “broods-at-risk” model based solely on the nesting dates supplied by the British Trust for Ornithology and estimating only the proportion of those nests that suffered toxicity exposure ratios greater or less than 5; and a “seasonal success” Markov chain model in which we estimated the number of chicks successfully raised by a typical female in the course of a breeding season. We then extended the “seasonal success” model, which described average individual reproductive success, to consider the potential effects of pesticide on populations and their long-term growth rates. In nearly all scenarios rooks were noticeably more sensitive to pesticide seed treatments than other bird species investigated. A principal cause is that rooks begin breeding earlier than the other species and are more likely to be breeding at a time when treated seeds are being sown. Temporal overlap was smallest for the yellowhammer and intermediate for linnets and skylarks. In all models, this is largely reflected in species vulnerability to pesticide effects with rooks faring worst, followed by skylark and linnet and with yellowhammer being least affected by seed dressings.

MO254

Dissipation of plant protection products from foliage - revisited

M. Ebeling, Bayer CropScience AG EnSaETXTV / Environmental Safety; M. Foudoulakis, Dow Agrosciences / RSGAACES; T.B. Fredricks, Monsanto Company / Zoology; I. Herrmann, BASF SE Agrarzentrum Limburgerhof; R. Murfitt, Syngenta Ltd / Environmental Safety; M. Wang, WSC Scientific GmbH / Dept Efate Modelling

In the environmental risk assessment for plant protection products in the EU according to the EFSA GD (2009), focal species representing herbivorous birds and mammals are often the potentially most exposed group, due to high food intake rates mainly related to relatively low energy content of their food. In the EFSA GD (2009) a generic DT50 of 10 days for dissipation of residues from spray applications on foliage is recommended to calculate multiple application factors (to account for residue accumulation owing to more than one application) and 21-d time-weighted average factors (to account for residue dissipation). As under the previous EU GD (SANCO/4145/2000, 2002) this generic DT50 of 10 days is recommended with reference to Willis & McDowell (1987) who evaluated 450 DT50 values for 81 chemicals for a broad spectrum of vegetative plant materials, resulting in mean DT50 values ≤ 5.9 days. In the meantime both the chemical spectrum of plant protection products and the regulatory assessment schemes have evolved. Therefore an evaluation of *ca.* 400 regulatory foliage residue dissipation trials for more than 30 active substances has been initiated, with the objective to verify the recommendations in the EFSA GD (2009) with more recent data generated under European field conditions.

MO255

Realism in freshwater field microcosms

C. Jenkins, HLS / Ecotoxicology; R. Jenkins, E. Hopkins, HLS; E. Quinton, P. Xirogiannopoulou, HLS / Aquatic Ecotoxicology and Biodegradation

In our microcosm studies, we have employed total enclosure methods to obtain more reliable, quantitative estimates of insect emergence and used emergence and reproduction endpoints in pesticide studies. Establishment followed published guidelines (HARAP, CLASSIC, and OECD) but after pesticide treatment, microcosms were covered by insect-proof enclosures which retained emerging insects and provided the opportunity for reproduction and re-colonisation. This

method generates more information about the life histories of the emerged insects and provides data that can more competently address the need to define the Regulatory Acceptable Concentration (RAC) for sensitive univoltine and semi-voltine species (EFSA, 2013). In this poster we compare the seasonal abundances of macro-inveretebrates with entirely aquatic life histories and the diverstity and abundance of insect species that emerged in order to identify some features that could be used to characterise "realistic" exposures, and to attempt to define factors that might impact on the determoination of the RAC. The numbers of many of the most abundant macroinvertebrates (Asellidae, Crangonyctidae and many Gastropods), increased substantially during the summer months reflecting the self-sustaining "realism" of the systems but these population increases and the variability often observed in replicate microcosms impacted both on the analysis of significance and the Minimal Detectable Difference (MDD, EFSA, 2013). A total of 121 species of insects (total number = 16,567) from the subfamilies of chironomids and 14 families of other insects were found of which over 15,000 were chironomids. The Occupancy Frequency Distribution (OFD) for chironomids was bimodal and similar to that reported by Raunkiær (1918). This naturally "realistic" distribution of species provides a basis for defining the levels (specied, genus, family) at which univariate analysis can be applied and the limitations that exist in identifying the RAC based on pooled data. The methods described here contribute to an understanding of how field microcosm data can be used to assess the effects of plant protection products and what limitations "realism" can place on defining the RAC.

MO256

Don't mix the grape and the grain: are ERA official procedures suitable for inorganic plant protection products?

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Most of currently used active ingredients in Plant Protection Products (PPP) are organic xenobiotics. Despite in numerical terms, natural inorganic compounds represent a small minority, their usage is extremely widespread. According to FAO data, inorganics represent about half of the amount of fungicides applied in Europe in the last decade. Due to the high number of xenobiotics registered as new substances (or re-registered after the previous authorization has expired) each year, most of the EU official procedures to assess the ecological risk posed by PPP are calibrated on these chemicals. However, the regulation is common for all substances used as pesticides, disregarding their chemical nature. This work tries to assess to which extent ERA PPP EU official procedures are suitable to describe the environmental fate and related effects on non-target organisms of natural inorganic substances using copper compounds as case study. Copper is an heavy metal naturally present in most environmental compartments. It is involved in biological processes and plays an important role as micronutrient for several organisms. Beside agriculture, where its use dates back to several decades ago, copper enters many anthropogenic activities determining several kind of emissions into the environment. Predictive models usually recommended at EU level were used to estimate environmental concentrations of copper following its use as fungicide according to good agricultural practice. Estimations for soil, surface waters and groundwater were compared to monitoring data retrieved from the literature. The results will be discussed in the light of the mechanistic parameters guiding the exposure models. Also, the effects on several organisms measured in different tests was critically considered to assess the relevance of some environmental parameters in determining both the bioavailability and the overall toxicity of copper compounds, bearing in mind that copper body concentration, as for all the micronutrients involved in biological processes, is regulated by homeostatic mechanisms. The use of models such as toxicokinetics-toxicodynamic models (TK/TD) or Biotic Ligand Models (BLM) can help in the evaluation of copper toxicity and bioavailability. Finally, some practical suggestions will be proposed to perform a proper ecological risk assessment of copper compounds within the framework of the EU PPP registration procedure.

Novel approaches to incorporate in vitro bioassays in risk assessment (P)

MO257

TOXICITY ASSAY USING LYSOSOMAL RESPONSES AND LYSOSOMAL PROTEOMIC APPROACH IN SACCHAROMYCES CEREVISIAE

N. Nguyen, Bioprocess Engineering; Y. Kim, Chungbuk National University / School of Life Science; J. Min, Chonbuk National University / Division of Chemical Engineering

This study assesses the toxic effects of two kinds of chemical (reactive oxygen species (ROS) and non-oxidative stress-making (NOSM) reagents) on the yeast *Saccharomyces cerevisiae*. Exposure of some organisms to oxidative stresses due to toxics alters lysosomal enzymes, thus we used intracellular lysosomes in *S. cerevisiae* as a biomonitoring tool to detect oxidative stresses by pesticides and heavy metals. Beside this, the effects of pharmaceuticals were evaluated simultaneously to examine NOSM reagents influence. The results indicated that each chemical has an optimal concentration at which the lysosomal response signal reaches the peak whereas the growth of yeast was not affected. It means that our method can detect the toxics at their sub-lethal doses. Additionally, the expressions of lysosomal enzymes of *S. cerevisiae* under the effects of these toxins were examined using two-dimensional gel electrophoresis (2-DE) method and provided a better awareness into toxic impact on cell, as well as screened some specific biomarkers for each toxic. These biomarkers were fused with different expressing fluorescent proteins to construct new recombinant yeasts which can detect specific toxicity or general toxicity.

MO258

Autonomous on-site monitor and laboratory determination of estrogenic activity in waste water using the Arxula adeninivorans yeast estrogen screen.

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Estrogenic substances are mainly introduced by waste water treatment plants into the environment and affect especially the aquatic environment already in very low concentrations. Based on PNEC values the European Commission derived Environmental Quality Standards for the natural 17?

MO259

Size-segregated characterization and toxicity of atmospheric particles near a cement plant in Catalonia, Spain

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Air pollution is composed by a complex mixture of substances that include volatiles and particulate matter. The breathable fraction of ambient particulate matter (PM) is often referred to as PM_{10}, defined as particles with a median aerodynamic diameter less than 10 µm, and it is a widely used air quality indicator. PM_{10} may further be divided into two main size fractions, a "coarse" fraction (2.5-10 µm) and a "fine" fraction (0.1-2.5 µm). The fine particulate matter $PM_{2.5}$ and PM_1 are able to go deeper in lung and alveoli, being related to cardiovascular and pulmonary diseases, as well as cancer. Due to its adverse impact on human health, ambient fine particulate matter ($PM_{2.5}$) is among the atmospheric pollutants subject to environmental regulation, particularly in the European Union. The problem of air pollution is especially important where dense urban populations are exposed to anthropogenic emissions. In such areas, the identification of the pollutant sources and a reliable estimation of their contributions to ambient particulate matter (PM) levels are necessary to set air quality improvement strategies. The aim of this study is to identify and characterize the size, chemical composition and toxicity of airborne particles in the vicinity of a cement plant in Catalonia, Spain. PM_{10}, $PM_{2.5}$ and PM_1 fractions were sampled and mineral matter (Al_2O_3, SiO_2, CO_3^{2-}, Ca, Fe, K, Mg, Ti and P), trace elements (As, Ba, Bi, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Ga, Gd, Ge, Hf, Hg, Ho, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Sr, Ta, Tb, Th, Tl, U, V, Y, Yb, W, Zn, Zr), C, TOC, N, S and soluble salts were analysed in the three different particles fractions. Moreover, the levels of ecotoxicity, cytotoxicity and genotoxicity were evaluated by using the photo-luminescent bacteria *Vibrio fischeri*, MTT and Comet Assays (both using human lung epithelial cells A549 as target cells), respectively. The results found in this study will help to quantify the contribution of cement plants as releasers of particle matter, with respect to other emission sources, as well as to characterize the toxicity of air particles and to assess the risks for the human health.

MO260

A rapid bio-assay to monitor humoral immunotoxicity

E.J. Pool, University of The Western Cape; K. Lategan, University of The Western Cape / Medical Biosciences

Introduction The immune system defends hosts against attacks by pathogens. The immune system consists of innate immunity, or the immune mechanisms that are already active at birth, and also acquired immunity which develops after contact with a specific pathogen. The acquired immunity consists of two defence mechanisms name cell mediated immunity, which fights against intracellular pathogens such as mycobacteria, viruses and cancers, and humoral immunity that defends the host against extracellular pathogens such as most of the bacteria and parasites. A very important characteristic of the humoral immunity mechanism is that antibodies are employed that specifically recognize the pathogen. Antibodies

are synthesised by B-lymphocytes. Hybridoma cell lines are B-lymphocytes fused with a cancer cell line (myeloma) to form a hybrid cell type that synthesises antibody and is immortal. The aim of the current study was to develop a humoral immunotoxicity assay employing hybridomas. Methods Hybridomas were incubated with different concentration of cadmium chloride ($CdCl_2$), a known cytotoxic chemical, for 24 hours. Magnesium chloride ($MgCl_2$) was used as a control metal salt with no reported adverse effects on cells. After the exposure period an aliquot of the culture medium was removed for antibody quantitation. The antibody concentration of the medium is indicative of antibody synthesis by the cells. Antibody synthesis was measured using an ELISA. Cells were then tested for cytotoxicity using the XTT assay. XTT measures mitochondrial metabolic processes. Results XTT results shows that $CdCl_2$ inhibits metabolic processes of the cells, while $MgCl_2$ has no effects. The effect of $CdCl_2$ was concentration dependent. The IC50 for $CdCl_2$ cytotoxicity is 14μM. The ELISA for antibody concentration shows that $CdCl_2$ inhibits antibody synthesis by the cells, while $MgCl_2$ has no effects. The effect of $CdCl_2$ was concentration dependent. The IC50 for $CdCl_2$ humoral immunotoxicity is 42μM. Discussion The results show that hybridomas can be used to monitor humoral immunotoxicity. In this study the assay was implemented to monitor heavy metal cytotoxicity and effects on antibody synthesis.

MO261

Hydrolytic enzyme activity of Daphnia magna and implications for rapid toxicity testing

P. Roslev, M. Orsted, Aalborg University / Biology and Environmental Science

Daphnia magna is a widely used model organism for aquatic toxicity testing. *D. magna* standard assays for acute toxicity tests target endpoint such as inhibition of mobility. However, use of this endpoint can be somewhat ambiguous and time-consuming.In this study, we investigated the extra- and intracellular hydrolytic enzyme activity of starved *D. magna* after exposure to organic and inorganic toxicants. In vivo enzyme activity was quantified using 15 fluorescent enzyme probes based on methylumbelliferyl fluorophores. Probing of *D. magna* enzyme activity was carried out after short-term (24-48 h) and long-term (21 days) exposure to different metals and organic toxicants including glyphosate (Roundup). Toxicant induced changes in fluorescence were compared to changes in mobility, survival, ATP content, and lipid biomarkers. The results showed that extra- and intracellular hydrolytic enzyme activity was quantifiable as changes in whole body fluorescence of *D. magna*, and as changes in fluorescence of the surrounding water. Juvenile and adult *D. magna* displayed a range of easily detectable enzyme activities including those of aminopeptidases, arylsulfatases, esterases, lipases, glucoside hydrolases, and phosphatases. Roundup was shown to affect hydrolytic target enzymes in *D. magna* including alkaline phosphatase. The results suggest that sublethal endpoints such as in vivo hydrolytic enzyme activity can be used as an index of *D. magna* fitness in bioassays. A combination of enzyme based endpoints and fluorescence measurements may be applied as a simple and rapid quantitative supplement for toxicity tests with *D. magna*.

MO262

Potential of serum-free cell based test systems – A case study on AhR-based bioassays

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The aim of reducing animal testing is common sense. But why do we researchers need fetal calf serum (FCS) for our cell culture instead of more reliable, sustainable, economical, safer, tailor-made and more ethical substitutions such as serum-free, chemically defined media (SFM/CDM)? This talk will elucidate the possibilities by using SFM/CDM in ecotoxicological assessments on basis of commonly used hepatocarcinoma/hepatoma cell lines. These cells were adapted to SFM/CDM as well as growth in suspension to uncouple the growth from adherance as well as invasive passage procedures. Therefore, the comparability of aryl hydrocarbon (Ah)-receptor activity based bioassays between traditional aherent as well as serum-free suspension cultures were investigated. Furthermore, several new methods were possible to perform. Suspension cultures could be applied to measure and monitor the cell vitality/viability, micronuclei formation as well as used in applications with need for passive dosing. In addition, chemical analytics towards complex metabolomic as well as proteomic studies are facilitated due to the low complexitiy of chemically defined media. The newly developed suspension cell lines HEPG2-S and H4IIE-S were capable to stably grow at cell densities up to 2.5 – 3.0?10^6 cells ml^{-1} and high viabilities (99 - 100 %) under uncontrolled batch culture conditions. The suspension cells were successfully applied in the Micro-EROD (HEPG2-S, H4IIE-S) bioassay with a comparability to serum-based, adherent test systems. Moreover, the working steps and costs could be reduced by using serum-free suspension cultures. The arguments given above prove that serum-free chemically defined media are comparable to traditional media containing ethically questionable FCS from newborn or unborn calves. Thus, our conclusion is that serum-free bioassays should help to increase the sustainability and public acceptance for *in vitro* bioassays.

MO263

Detection of endocrine effects by anthropogenic micropollutants in drinking water

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The joint project "Tox-Box – Risk assessment of anthropogenic micropollutants to assure the drinking water supply" (BMBF FKZ 02WRS1282I) aims for a harmonized, hierarchic test strategy to assess the toxicity of micropollutants that may occur in drinking water by means of the health-related indicator value (HRIV concept). This concept offers five health-related threshold values (≤ 0.01 µg L^{-1} to ≤ 3 μg L^{-1}) depending on availability and completeness of toxicological data, regarding genotoxicity, neurotoxicity and germ cell damages. As one part of the module "endocrine effects" within this project the Institute for Environmental Research at RWTH Aachen University will analyze and establish endocrine activity as an important, additional toxicological mode of action within this concept using a set of bioassays. The *in vitro* ERα/ AR CALUX® assay (Estrogen-/ Androgen-Responsive Chemical-Activated Luciferase gene eXpression) detects receptor-mediated endocrine activity in the human osteosarcoma cell line U2-OS (± S9 fraction for metabolic activation). A second *in vitro* assay, the H295R steroidogenesis assay, identifies alterations in the steroidogenesis (hormones 17β-estradiol and testosterone) with a competitive ELISA and effects on the expression of different steroidogenic genes (CYP11A, 3βHSD2, CYP17A and CYP19A) using quantitative real-time PCR in the human adrenocortical carcinoma cell line H295R. The *in vivo* reproduction toxicity assay with the mud snail *Potamopyrgus antipodarum* provides data on endocrine disruption at an individual as well as population level. First results indicate that benzo[*a*]pyrene, diclofenac and sulfamethoxazol, tris (1-chloro-2-propyl) phosphate (TCPP), atrazine, 2,4-dichlorophenol (2,4-DCP), tributyltin oxide (TBTO), diatrizoic acid (DTA) and perfluoroctanoic acid (PFOA), applied at their water solubility limit, have no affinity to the estrogen and androgen receptor without metabolic activation. The tests with S9 fraction are currently ongoing. Atrazine, 2,4-DCP and TBTO caused effects on hormone production in the H295R assay, which can be correlated with changes in the expression of steroidogenic genes. Results from the entire Tox-Box project, which is part of the BMBF funding measure "Risk Management of Emerging Compounds and Pathogens in the Water Cycle (RiSKWa)" will be used to establish a new guideline regarding the risk assessment of anthropogenic micro pollutants in drinking water.

MO264

Employing probabilistic hazard assessment approaches to examine high throughput in vitro toxicology datasets

D.A. Dreier, Baylor University / Environmental Science; K.A. Connors, Baylor University / Institute of Biomedical Studies; B.W. Brooks, Baylor University / Dept of Environmental Science

Although toxicological data exists for some environmental contaminants, little to no data exists for a vast number of chemicals. In order to assure the safety of these compounds, how can we utilize known toxicological data to predict the environmental and human health impacts of untested chemicals? And how does one select an assay for assessing chemical safety when multiple models systems exist? To examine these questions, we utilized chemical toxicity distributions (CTDs) to perform probabilistic hazard assessments (PHAs) of data from *in vitro* assays. These assessments allowed us to predict the likelihood of chemical classes eliciting specific toxicities at or above environmentally relevant concentrations, which can be used to examine several toxicology questions. For example, PHAs have the potential to predict toxicological properties of similar chemicals, identify thresholds of toxicological concern, prioritize the most problematic chemicals for additional toxicity testing, and identify characteristics for sustainable molecular design. In particular, this approach may be useful to understand endocrine disrupting chemicals. While these compounds have received extensive study in recent years, most environmental contaminants lack toxicity data for these endpoints. An initial PHA examined the comparative sensitivity of three *in vitro* assays for estrogen agonists representing a diverse group of industrial chemicals and pesticides from U.S. Environmental Protection Agency's Phase I ToxCast dataset. CTDs were compared at the 5th centile for all compounds, common compounds, and organophosphate compounds amongst available in vitro assays. When all available data were compared, the NCGC Agonist assay was the most sensitive for estrogenicity as there was a 5% probability of detecting a compound that will elicit an estrogenic response at or below concentrations of 0.089 mg/L. When only common compounds and organophosphate compounds were

considered, the Attagene CIS assay was the most sensitive assay with concentrations of 0.13 mg/L and 1.8 mg/L, respectively, at the 5th centile. We then extended this PHA approach to examine additional mechanisms of action and data from the ToxCast program.

MO265

Effect-based tools for monitoring (xeno)estrogens in surface waters: Variability and reproducibility of sample preparation and five different in vitro assays

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In vitro bioassays are increasingly used to assess estrogenic activity of environmental water samples and have been suggested as suitable tools for monitoring estrogenic contamination of surface waters. Such assays are of particular use as they measure the overall estrogenic activity of a sample, including the potent steroids 17β-estradiol (E2) and 17α-ethinyl estradiol (EE2); proposed annual average Environmental Quality Standards (AA-EQS) are 400 and 35 pg/L, respectively. For most routine chemical methods these EQS are below analytical limits of quantification, resulting in difficulties for monitoring E2 and EE2 under the watchlist mechanism of the Water Framework Directive. The use of sensitive *in vitro* assays could circumvent current detection problems by measuring the total activation potential of estrogenic substances in environmental samples by expressing overall estrogenicity in E2-equivalents (EEQs). It has been shown, however, that different assays lead to different EEQs for the same sample. Reasons for these differences are known, for example sensitivity differences of the assays towards certain substancesbut it remains unclear how to use and interpret bioassay results. Hence the aims of this study are to (1) compare EEQs of reconstituted water samples and extracts assessed by five commonly used bioassays in order to determine the variability and reproducibility of the assays and the sample preparation method (solid phase extraction) and (2) get insights into their validity for environmental monitoring. Initial results for three different bioassays show that inter-day reproducibility of derived EEQs varied between 2.5 and 30%. Comparison of the results from different *in vitro* assays showed that all assays were able to correctly detect the EEQ of the positive control. Only the ER-CALUX® was able to derive EEQs close to the calculated EEQs for the reconstituted samples. The 10x concentration difference between the two reconstituted samples was detected by the ER-CALUX®and the T47D-Kbluc. The YES underestimated the estrogenic load of these samples, whereas the T47D-Kbluc overestimated the EEQs of both mixtures. Data from the MELN and GeneBLAzer ER assays are yet to be analyzed. Overall, our findings suggest that *in vitro* bioassays are comparable to chemical measurements regarding variability and reproducibility of the derived EEQ concentrations.

Plants and pollutants in the environment (P)

MO266

Efforts to balance representativeness and feasibility: tests on two Myriophyllum species

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A number of studies have assured us that the use of additional aquatic macrophyte species in ecotoxicological risk assessment is reasonable in cases when standard *Lemna* test is either not applicable or sensitive enough (e.g. sediment toxicity, chemicals with specific modes of action). In search for the most suitable test design and species, one of the several tests and protocols developed is a growth inhibition test with *Myriophyllum* species in a water-sediment system. This study has focused on two *Myriophyllum* species – *M. spicatum* and *M. aquaticum*. Since there are some specific differences in the biology and ecology of the two species,it is beneficial to assess their overall suitability for use in refined risk assessment. Tests with three herbicides of well known mode of action (atrazine, isoproturon and 2,4 D) were carried out with small adjustments to the recently developed OECD guideline: the idea was to identify whether the different periods for adaptation/rooting phase are necessary, or the 3 day adaptation period is sufficient for both species. Also, the exposure phase for both species was 7 days. Standard OECD *Lemna* tests with the same test substances were run simultaneously for comparison. According to EC_{50} values based on plant length above sediment, two *Myriophyllum* species showed comparable sensitivities to atrazine, isoproturon and 2,4 D respectively (*M. aquaticum*: 0.317, 0.547, 0.1607; *M. spicatum:* 0.1949, 0.273, 0.591 mg/l). Apart from comparison of two species' relative sensitivity, the objectives of the study were to a) evaluate the sensitivity and reliability of various endpoints including whole plant length, fresh and dry weight and root weight vs. length above sediment which is a principle endpoint proposed by the guideline; b) compare relative growth rates of plants in adaptation, as well as in exposure phase; c) consider the advantages and disadvantages of each species based on sensitivity, ease of laboratory manipulation and representativeness (acknowledging the differences, e.g. submerged/emergent, native/non native) and d) estimate the applicability and added value of the additional test with aquatic macrophyte in water-sediment system, with either of the two *Myriophyllum* species, for ecological risk assessment. This study was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia via Grant No. 173037.

MO267

Myriophyllum biotests

G. Gonsior,

Up to know ring-tests with *Myriophyllum spicatum* in an unsterile water-sediment or axenic sediment-free system were performed. Both test designs are submitted for OECD evaluation. Most studies will be conducted based on the OECD Draft Guideline: Water-Sediment *Myriophyllum* sp. Toxicity Test based on Draft AMRAP Method: `Growth Inhibition Test for the Rooted Aquatic Macrophyte, *Myriophyllum* sp.` submitted to OECD for Evaluation, 22 July 2013. However there is no final and internationally accepted testing guideline available up to now and still some open questions for risk assessment have to be solved. The main focus of discussion is on the role of the roots and if they have any significant influence on the toxicological endpoints. To evaluate the root and the shoot separately should be treated with caution. An effect on one part of the plant might result in unclear findings compared to another part of the plant. The contribution will present data of total plant biomass in comparison to the draft guideline recommendation which only evaluates the shoot biomass. Further, data with three plants per replicate were generated and compared with the single plant per replicate approach.

MO268

Glyceria biotest

G. Gonsior,

Some plant protection products and industrial chemicals show an unavoidable high risk for aquatic plants. Due to the fact that *Lemna* only reflects the risk assessment of free-floating aquatic plants there might be a need for the establishment of other aquatic macrophyte systems. Up to now ring-tests with *Myriophyllum* spec. in an unsterile water-sediment or axenic sediment-free system were performed. However, it is still under discussion, if *Myriophyllum* and *Lemna* species could cover all questions for doing refined risk assessment on macrophytes. Tests with *Glycria maxima* are under discussion and could be performed in addition to reduce uncertainties. A water-sediment test with *Glyceria maxima* was further developed and results will be presented.

MO269

Effects of water- and sediment-exposure of linuron on Glyceria maxima

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The aquatic guidance document mentions the aquatic emergent *Glyceria maxima* (monocot) as one out of three obligatory standard test species that is required for the effect assessment of a pesticide with a herbicidal mode of action. It is advised to test *G. maxima* in case *Lemna* sp. is not sensitive or where there is expected uptake by the roots of sediment-rooted macrophytes and the herbicide primarily affects terrestrial monocots. Effects from sediment exposure to aquatic macrophytes have hardly been studied up to now. The recently submitted and ring-tested standardized OECD test-guideline for testing of *Myriophyllum* sp. gives a possibility for testing of sediment-exposure, although a method is lacking. Therefore, a toxicity test was undertaken including a comparison of exposure via the water layer and via spiked sediments in a static test-design following the OECD protocol. *G. maxima* shoots, originating from a GLP-certified culture, were adapted to laboratory conditions for a two days. Shoots were standardized to 2 to 3 leaves and one shoot attached to a rooted, 1 cm root-stock. Shoots were planted in a flower pot for pre-growth during one week. Smart and Bark growth medium and artificial OECD sediment with nutrients were used as standardized media. After this pre-treatment period, plant pots containing two shoots were exposed to five concentrations of linuron (0.5, 5, 50, 500, and 1000 µg/L) in the overlying growth medium of Smart and Barko for 14 days. For sediment-exposure, linuron was dosed in the sediment at high and low levels of 15762, and 8485 µg/kg (dry weight) respectively, and compared to a demi-water dosed control sediment exposure. In order to obtain sediment that is in equilibrium with pore water and the overlying water column, sediment was spiked

and shaken for 5 days with 0.75 L of Smart and Barko medium. After this shaking procedure, sediment particles were enabled to settle down, and removed to a flower pot and planted with two pre-grown *Glyceria* shoots. Pots were submersed in the equilibrated test solution. Linuron was measured in overlying water layer weekly and in shoots at day 14. Uptake of linuron by shoots and toxicity to plants was compared for sediment- and water-exposure test designs. At day 14, 34 – 39 % of Linuron concentrations was left in the overlying water layer when dosed in the water. Remaining concentrations were higher when dosed in sediment (55 – 59 %). Plant uptake from sediment was also higher.

MO270

A proposed ring-test protocol for an emergent macrophyte, Glyceria maxima, in a water-sediment system

J. Davies, Syngenta / Environmental Safety; M. Dollinger, Bayer CropScience

Under EU pesticide regulation, regulatory tests are required for the aquatic macrophyte, *Lemna*, and two algal species for herbicides. In 2008, participants of the SETAC – AMRAP (Aquatic Macrophyte Risk Assessment for Pesticides) workshop identified the need for additional regulatory tests for some herbicidal compounds where root uptake from sediment is considered an issue or where the sensitivity of standard algae and *Lemna* species is believed not to be representative of other macrophyte species. Consequently, new data requirements introduced under EU Directive 1107/2009 stipulate that further tests may be required for compounds which show selectively higher toxicity to either dicotyledonous or monocotyledonous plant species in terrestrial plant tests. In these cases, the recommended dicot and monocot species are *Myriophyllum* and *Glyceria*, respectively. A Draft OECD Test Guideline is currently under review for *Myriophyllum* species growing in a water-sediment system. The general principles of this test system are applicable to many aquatic plant species. This presentation will describe adaptation of this protocol to facilitate testing of the emergent, reed grass, *Glyceria maxima*. Data will be presented to demonstrate likely test performance and inform the test design. This proposal will form the basis of a test protocol that will be open for international ring-test from 2014 onwards.

MO271

Development and validation of a stock culture independent duckweed microbiotest with Spirodela polyrhiza

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A "stock culture free" duckweed microbiotest has been developed, based on the use of "vegetative dormant stages" which are produced by only a few duckweed species. These dormant stages (named turions) can be stored and germinated at the time of performance of the bioassay. *Spirodela polyrhiza* is a duckweed species closely related to *Lemna*, and its turions germinate in a few days of time, and then immediately develop a first frond. A simple and practical 3 days microbiotest has been worked out in a multiwell test plate with measurement of the area of the first frond of germinated *Spirodela polyrhiza* turions as effect criterion. The area measurements are made by image analysis on a photo of the test plate taken at the end of the exposure time. The sensitivity of the *Spirodela polyrhiza* microbiotest has been determined for inorganic and organic chemicals, and the 72h EC50's for 16 compounds have been compared with the 7 days EC50's of the *Lemna* toxicity test. The data pair comparison revealed that the alternative duckweed microbiotest has the same sensitivity as the conventional *Lemna* bioassay ($R^2 = 0.97$). A preliminary ringtest has recently been organized with participants from 6 countries, with performance of the new assay on the reference chemical KCl. The preliminary ringtest revealed that all the participants obtained very similar 72h EC50's with a mean value of 6.593 mg/l KCl and a variation coefficient of only 6.19%. An International Interlaboratory Comparison is now in progress, with a large number of participants from many countries, to assess further the precision and the robustness of this promising practical and low cost "stock culture free" duckweed microbiotest.

MO272

A novel bioassay using root re-growth in Lemna

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A new phytotoxicity test method based on root elongation of three Lemna species (Lemna gibba, L. minor, and L. paucicostata) has been developed. Tests with aquatic plants have, typically, favored measurements on fronds (e.g. frond number, area, biomass) rather than on roots, due, in part, to issues associated with handling fragile roots and the time-consuming procedures of selecting roots with identical root lengths. The present method differs in that roots were excised prior to exposure with subsequent measurements on newly developed roots. Results show that there were species-specific difference in sensitivity to the five metals tested (Ag, Cd, Cr, Cu and Hg), with Ag being the most toxic (EC50=5.3-37.6 µgL(-1)) to all three species, and Cr the least toxic for L. gibba and L. minor (1148.3 and 341.8 µgL(-1), respectively) and Cu for L. paucicostata (470.4 µgL(-1)). Direct comparisons were made with measurements of frond area, which were found to be less sensitive. More generally, root re-growth was shown to reflect the toxic responses of all three Lemna species to these five important metals. The root growth bioassay differs from three internationally standardized methods (ISO, OCED and US EPA) in that it is completed in 48 h, the required volume of test solutions is only 3 ml and non-axenic plants are used. Our results show that the Lemna root method is a simple, rapid, cost-effective, sensitive and precise bioassay to assess the toxic risks of metals and has practical application for monitoring municipal and industrial waste waters where metals are common constituents.

MO273

A novel bioassay using the lettuce root elongation

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The root elongation test is one of the simplest methods of environmental monitoring in terms of simplicity, rapidity and economy since it merely employs filter paper, distilled water and Petri dishes. However, the support used for the test, filter paper, is known to be problematic since toxicants, metal ions in particular, are easily absorbed, thus causing an interference with the toxicity of the same metals. Our new method is the same as the conventional root elongation method (US EPA filter paper method) in that root elongation is an endpoint, but differs from the method since no support including filter paper is employed, and exposure time is shorter (48 h in this test versus 120 h in the US EPA test). In our root elongation test, seeds are added and floated on the surface of control and test liquid samples in Petri dishes. After a 48 h culture period, the root length of seeds is measured with an image analyzer. EC_{50} and CV values are then calculated for each respective test samples. The 6-well seed floating method has higher sensitivity to metals than the conventional root elongation method although the sensitivity to VOCs is similar or lower than the US EPA method. It can be temporarily concluded that the new root elongation method is simpler, quickerand more economic than the US EPA method at least in response to pollutants.

MO274

Chlorophyll Spectrofluorescence Measurements of Healthy Maple Leaves and With Fungal Infection during Growing Season

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Emission fluorescence measurements and absorption spectra are successfully used for rapid diagnostics of the functional activity of the plants photosynthetic apparatus. These methods allow to calculate concentration of leaf pigments and to find changes in the photosynthetic apparatus at the earliest studies of external influences. Photochemical reactions in living objects can be studied with the only indicator chlorophyll. However, the influence of pathogenic fungi on fluorescence and contents of chlorophyll has not been studied enough with these methods. Norway maples (*Acer Platanoides L.*) grow in central Europe and southwest Asia and widely used as ornamental plants. Its grooving season lasts from the end of April till the end of October. However, in the recent years maple plantings have affected with fungal diseases like tar spot (*Rhytisma acerinum*). Tar spots is a plant pathogen that commonly affects sycamores and maples in late summer and autumn so there form looks like tar. Freshly harvested maple leaves from trees growing at the campus of Moscow State University were studied in the spring and during autumn color change, both healthy and with tar spots. Fluorescence spectra were measured using a spectrometer Solar. F685/F740 ratio of intensities at 685 and 740 nm was calculated for them. The concentration of chlorophylls was measured in ethanol extracts using Unico 2804 photometer. Chlorophyll concentration distribution on the surface of the leaf was plotted. Ratio F685/F740 sharply decreases at the beginning of May, then it monotonically decreases to September, then it is practically constant for healthy green leaves. Ratio F685/F740 monotonically increases with decreasing of chlorophyll concentration during autumn color change. Ratio F685/F740 is higher for leaves with fungal diseases with the same pigmentation. Ratio Chl *a*/Chl *b* of the concentration chlorophylls *a* and *b* was calculated and equals 2.38 for all the leaves during the autumn color change both healthy and with fungal diseases.

MO275

PCB concentrations in soil and leaves along four transects from a contaminated site in Northern Italy

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PCBs are a class of organic compounds widely used in the industry until the hazard posed to both the environment and human health by their use became evident. Due to their properties, PCBs are highly persistent in the environment and tend to accumulate in the food chain, posing adverse effects on human health and the environment. PCBs are industrial chemicals which were massively produced in many countries for many decades until measures were taken to reduce their release to the environment, resulting in a substantial decrease of environmental levels of these compounds. However PCBs are still present in the environment and can be released from reservoir compartment, such as contaminated sites. The area of Brescia, a highly industrialized city in the Lombardy Region (Northern Italy) is characterized by the presence of an industrial plant which produced PCBs between 1930 and 1984. Some areas surrounding the plant were found to be heavily contaminated, with high concentrations in air, irrigation water, soils to such an extent that the area was declared "national high contaminated site" by the Italian authorities. In the last two decades, different studies were conducted in this area to investigate PCB contamination of drinkable water, human blood, soil and forage, human adipose tissue, domestic animals, animal feed and air. Therefore the aim of the present study was to investigate the potential of the contaminated area in driving the PCB contamination in the surrounding areas. Four sampling campaigns were organized to collect samples of soil and leaves at increasing distance from the former PCB production plant along four 100 km transects. Samples were analysed with GC-ECD and GC-MS and the results of each transects were compared in order to evaluate the presence of a contamination gradient, that decrease with increasing distance from the point source. The fingerprints of the soil and leaf samples were compared to profiles of Arochlors and profiles of soils from the contaminated site in order to understand if the concentrations found in the transect samples could be attributed to the Brescia site. In order to interpret the actual soil and leaf concentrations and predict the order of magnitude of fluxes, a number of simulations were performed using SoilPlusVeg, a dynamic air-vegetation-soil model. This study confirms the importance of such a type of sampling campaign to demonstrate the impact of this PCB production plant also on the surrounding areas.

MO276
Removal of Pharmaceuticals and Personal Care Products in Mesocosm Constructed Wetlands
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A large variety of pharmaceuticals and personal care products (PPCPs) are usually present in urban wastewaters, and since common WWTPs are not able to efficiently remove all PPCPs, many of them are released into surface water bodies. Technologies to remove PPCPs discharged into receiving waters like ozonation, reverse osmosis, and advanced oxidation processes can lower the level of pharmaceuticals but are extremely expensive. There is a growing interest in using constructed wetlands (CWs) as a low impact and economical alternative to treat contaminated waters. CWs remove contaminants through a series of complex physical, chemical and microbial interactions which involves a variety of processes including biodegradation, sorption, sedimentation, microbial and plant uptake. Though CWs has shown the ability to remove some PPCPs, the mechanisms involved are largely unknown. In this study, six mesocosm-scale CWs of different configurations are operated to assess their removal ability of PPCPs. The CWs differ in some design parameters, namely the presence or absence of plants, their species (*Thypha augustifolia vs Scirpus mucronatus*) and the presence or absence of soil matrix. Spiked tap water is used to mimic the urban wastewater. The efficiency comparison of different CWs is achieved by analyzing mass removal. The different behavior of each CW regarding the removal of selected PPCPs is monitored in order to suggest a more efficient type of wetland. The effect of different CW configurations on removal of every PPCP is also studied in order to propose elimination patterns. Generally, the presence of plants enhanced the removal of PPCPs and the rather efficient removal suggests that CW systems to remove selected pharmaceuticals with minimal land requirement may be practical in tropical regions.

MO277
Toxicity of veterinary medicinal products: comparison between terrestrial and aquatic macrophytes
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The different responses of aquatic and terrestrial macrophytes as experimental model organisms for the ecological risk assessment of veterinary medicinal products (VMPs) has been investigated. VMPs are used in large quantities, but the assessment of associated risks to the environment is limited although they might reach the environment via spreading onto agricultural soil or directly in water. This project presents the results of tests performed with *Myriophyllum aquaticum* and ten species of terrestrial plants using a thiamine analogue acting as antiprotozoal agent upon Coccidia parasites in chickens and cattle. The terrestrial plants were tested for seedling emergence and vegetative vigour, according to OECD 208 and OECD 227 respectively. The terrestrial species were selected in order to represent both monocotyledon and dicotyledon crops. *M. aquaticum* was chosen as the representative aquatic macrophyte. The method followed was a grown test performed in a sediment-water-system developed to assess toxicity of substances via the water-phase and sediment-phase (method described in Maltby and al. 2010), called "AMRAP-test". The aim of this project was the comparison of aquatic and terrestrial plants responses to the veterinary medicinal product tested. Moreover these results were preliminary evaluated in order to better understand if these differences (if any) are due to testing schemes, application patterns or intrinsic species sensitivity.

MO278
Toxicity of Molybdenum to the aquatic floating macrophyte Lemna minor
D. Bowes, P.K. Sibley, University of Guelph / School of Environmental Sciences
Extraction of metal resources in Canada is increasing, particularly in northern and sub-Arctic regions. A significant challenge in these often harsh environments is the restoration of mine sites that may contain elevated concentrations of a number of elements. As part of a larger project to investigate potential restoration techniques, including phytoremediation, for uranium mine sites in northern Saskatchewan, we investigated the toxicity of molybdenum, which is frequently detected at elevated concentrations in run-off and leachates from the mine waste piles on the aquatic plant *Lemna minor*. At present, there is little information on the toxicity of Mo toward aquatic plants. Toxicity was assessed in a series of standard 7-day growth tests using concentrations ranging from 0.1 to 1000 mg/L, selected to encompass concentrations of Mo potentially encountered in mining site run-off and leachates, and to define toxicological thresholds for the suite of endpoints evaluated. Toxicity was assessed by measuring changes in wet/dry mass, growth rate, and photosynthetic activity. EC50 values for these endpoints were, respectively, 73.6, 167.9, 372.7, and 407.8 mg/L. Corresponding EC25 values were 19.1, 46.0, 163.3, and 64.7 mg/L. Using the EC25 value for the most sensitive endpoint (wet mass), and the highest concentration of Mo detected at the mine site (6.1 mg/L in waste pile leachate), a HQ value of 0.32 was estimated. Collectively these data indicate that Mo poses minimal risk to naturally occurring aquatic macrophytes potentially exposed to mine waste run-off and plants potentially used in phytoremediation efforts. However, additional studies are planned to assess the effect of variable pH and hardness on Mo toxicity as both factors can influence the toxicity of this element.

MO279
Effect of Bilge Water on Mitosis and Antioxidant Enzyme Activities in Allium cepa L.
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In this study, the potential cytotoxicity and genotoxicity of the bilge water was investigated using the *Allium cepa* aceto-orcein squash method. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities were assessed on root-tips of *A. cepa* L. exposed to the wastewater samples. Physicochemical analysis revealed appreciable amount of lead, chromium, zinc, manganese, cadmium, iron and copper. Compared to the control (tap water), treatment with the wastewater resulted in statistically significant ($p < 0.05$) inhibition of root growth and decrease in mitotic index with increasing concentration. Chromosomal aberrations induced in the onion root tip cells were mostly sticky chromosomes, laggards, vagrants and bridges. SOD, CAT and GSH-Px enzyme activities decreased in the common onion root tip cells indicating high rate of genotoxicity. The findings reveal that the toxic chemicals in the wastewater are responsible for the observed genotoxic effects by oxidative damage on the onion root tip cells. **Key words**: chromosome aberration; mitotic index; bilge water; antioxidative enzymes

MO280
Risk assessment of the use of dewatered and composted sewage sludge as soil amendments: behavior of metals in soils and their uptake by plants
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The production of sewage sludges in urban wastewater treatments plants (WWTP) is a growing environmental problem. The use of such sludges as organic soil improvers seems an attractive possibility, because it would enable valuable components, such as organic matter, N, P, K and other nutrients to be recycled. However, this practice represents a potential risk to the environment, because of their possible high heavy metal content, a problem that may be aggravated if the metals are mobilized in the soil, accessible to be taken up by plants or transported in drainage waters. Plant accumulation and trophic transfer may result in exposure of animals, including humans, to these contaminants. The aim of this study was to evaluate the potential risk of the use of dewatered and composted sewage sludge as soil amendments; their mobilization is soils and their uptake by plants. For this purpose, sewage sludge from two different WWTP (SS1 and SS2), and composted sewage sludge with agricultural wastes (SSAWC) were used. Three different application rates were tested, 6, 12 and 24 ton dry matter/ha, using three replicates per treatment, in 3 kg pots, subjected to outdoors conditions. Pots were cultivated with a hybrid variety of sorghum and Sudan grass (*Sorghum bicolor* x *Sorghum sudanense* var. Rocket) 8 d after the incorporation of the amendments. The effects of the application of dewatered and composted sewage sludge were assessed 60 d after sowing, evaluating: (i) soil properties: pH(H_2O), electrical conductivity, organic matter content, and nutrients (N, P, K, Ca and Mg); (ii) total, effective bioavailable, and potentially bioavailable heavy metal content in soils (Cd, Cr, Cu, Ni, Pb and Zn); and (iii) plant properties: biomass production, foliar area, chlorophyll content, and heavy metals content (Cd, Cr, Cu, Ni, Pb and Zn). Both sludges had a beneficial effect on pant production and properties, without a significant increase in total heavy metals concentration, both in soils and in plants. Only Zn concentration in plant tissue rose as a consequence of SS2 application. Accumulation factors for Cr, Cu, Ni and Zn in plants were low, and their concentrations in the plant were lower than the maximum tolerable level for cattle. However, it is noticeable the increase in the potentially bioavailable pool of some metals in soils, which importance as a risk evaluation criteria was discussed in this study.

MO281
Effects of lead and salt-induced stress on germination, proline accumulation and soluble proteins concentration in wheat (Triticum aestivum L.)
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The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil pollution (water stress), nutrition imbalance, specific ion effect (salt stress), or a combination of these factors. Heavy metal toxicity induces secondary oxidative stress by importing the formation of harmful reactive oxygen species. Under condition of salt stress, proline accumulation serves as a defense against osmotic challenge by acting as a compatible solute. Proline has also been demonstrated to scavenge hydroxyl radicals and singlet oxygen, thus providing protection against reactive oxygen species induced cell damage. The aim of this study was to assess the effect of lead and salt-induced stress on germination, proline accumulation, and soluble protein concentration in wheat (*Triticum aestivum*). Wheat seedlings were exposed to 50 and 100 mM NaCl, and 20, 50, 200 and 500 ppm $Pb(NO_3)_2$ for four days. Root and coleoptile lengths were measured, as well as proline and soluble protein concentrations. High lead concentrations inhibited apical dominance, as the lateral roots were longer than the primary roots in lead-treated seedlings. The inhibition of root growth might be a result of Pb-induced inhibition of cell division in root tips. Proline concentration in plants increased with increasing Pb concentration in the medium. Proline accumulation was stimulated under salt-induced stress, due to osmotic stress caused by high NaCl concentrations. Increased concentrations of NaCl and Pb in growth media also resulted in an increase in proline accumulation in plants, with a synergistic effect between the two compounds. The level of soluble proteins increased upon exposure to high lead concentrations, which may be a result of oxidative stress induced by Pb uptake.

MO282
Accumulation of nickel and zink by Thlaspi kovatsii and T. praecox populations from the ultramafics of Serbia
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Species of plant genus *Thlaspi* L. are well known for their capacity to aquire and accumulate heavy metals from soil. For this study we collected samples of *Thlaspi praecox* Wulfen and *T. kovatsii* Heuffel from 5 ultramafic sites in Serbia. We analysed the content of macronutrients (P_2O_5, K_2O, Ca, Mg, Fe) and selected trace elements (Ni, Zn, Mn, Cu, Cr, Cd and Pb) in soil samples and in both roots and shoots of collected plant material. The analysis showed features of accumulations typical for the ultramafic soils, with low contents of P_2O_5, K_2O and Ca and high concentrations of Mg and Ni. High available nickel concentration was found in all analyzed soil samples (152 – 506 mg kg^{-1}), with ratio of available concentrations in soil versus shoots of 1:4 up to 1:32. High contents of nickel were found in all analysed plant samples with an exception of plants from Rogozna Mountain (60 – 14000 mg kg^{-1} shoots dry weight and 48 – 3930 mg kg^{-1} roots dry weight). The highest nickel concentration was found in *T. praecox* shoots collected at Maljen Mountain (locality Crni vrh). High zinc content was also detected in the roots and shoots of the studied species (concentrations of 75 to 897 mg kg^{-1} in dry weight of roots and 87 to 1086 mg kg^{-1} dry weight of shoots). Exception was once again the plants collected at Rogozna Mountain, where a statistically significant lower concentration of zinc was found both in roots and shoots of collected plants. Statistically significant differences in magnesium, zinc and iron contents in the roots and shoots were detected among studied species, with higher values of these elements in *T. praecox*. There was no significant difference in nickel concentrations between studied species, although we detected extreme values of nickel content in *T. praecox* at Maljen Mountain. There is a clear variability in detected heavy metal accumulation rates among studied populations. Further *in situ* and *ex situ* studies are needed in order to evaluate the main causes of found variability and the extent of accumulation potential of the studied species, with special regard to the nickel accumulation in *T. praecox* populations at Maljen Mountain. Obtained data would be useful to evaluate the advantages of using this species as an experimental model in biotechnology and metal phytoextraction.

MO283
Uptake of dioxins and polycyclic aromatic compounds from field-contaminated soil by zucchini (Cucurbita pepo)
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Zucchini is a plant belonging to the *Cucurbita* genus, and it has been shown to accumulate organic pollutants from soil. In this study, zucchini (*Cucurbita pepo*) was grown on soil from two contaminated sites. One site was contaminated with polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs, also known as dioxins), and the other site with polycyclic aromatic compounds (PACs). PACs include the well-known soil pollutants polycyclic aromatic hydrocarbons (PAHs), but also more polar PACs such as the oxy-PAHs and azaarenes. These substances are produced in the same processes as PAHs, but can also be formed when PAHs degrade in the environment. The oxy-PAHs and the azaarenes are generally more polar than the PAHs, and may therefore display different behavior in contaminated soils, for instance in plant uptake. The contaminants included in this study were the seventeen 2,3,7,8-substituted dioxins, 16 PAHs, 11 oxy-PAHs and 4 azaarenes, thus covering substances that differ substantially in hydrophobicity and other physicochemical properties. The aim of the study was to determine the uptake by zucchini and to investigate if the uptake varied between the different soils and between the different contaminants. We also investigated to what extent the uptake correlated to soil parameters, for instance organic carbon, black carbon, nitrogen and phosphorous.

MO284
Water hyacinth infestation, an ecosystem service and not a pest? A periurban agricultural zone case study.
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Mexico City is located at the mexican basin once a system of lakes fed by springs and rivers from the surrounding mountains. Nowadays, the Xochimilco aquatic system is part of what remains of that great lake system and is a complex network of agricultural areas (named chinampas) surrounded by canals, covering an area of only about 15% of the original lake. Its aquifers have been exploited as a water source for Mexico city andto avoid complete dryness of the Xochimilco lake untreated seawage and treated wastewater have been dumped into the canals since 1970. Nevertheless it is still an important agricultural area that uses the canal´s water for irrigation of vegetables, flowers and other crops. The high agriculture activity of the zone has impacted the aquatic system with pesticides pollution from, which is a hazard for the edible crops and the regional fauna as has been demonstrated in endemic amphibians. Therefore achieving good water quality in the canals is of great importance. Several processes have been reported in the literature for the removal of pesticides but in recent decades, seeking to minimize costs, new technologies have been developed for the removal of pollutants. These technologies called Emerging Technologies focus more on low scale processes or on local treatments in isolated populations. Some of them adapt conventional methods, such as adsorption with more affordable materials, such as the water hyacinth, which is considered to be bio-adsorbent and an attractive phytoremediation agent. Water hyacinth (*Eicchornia crassipes*) has demonstrated an amazing ability to absorb and concentrate a range of pollutants. The Xochimilco canals suffer from water hyacinth infestation, which is constantly removed and is regarded as a pest. However, it can absorb organic contaminants present in the canals as it has been demonstrated for metals. In the present study we established that water hycianth´s pesticide (organophosphates and organochlorides) bioaccumulation and translocation rates makes it a suitable phytoremediator of pesticides. Our study demonstrated that water hyacinth can be used as a water biopurifier and therefore its management should be done as a plant offering

environmental services (decontamination) rather than a weed. We discuss how appropriate management can increase the environmental service of the water hyacinth.

MO285

How to assess the risk of multiple applications from single application studies

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How multiple applications of a pesticide are considered in the risk assessment is one of the critical points in the course of the ongoing revision of the Guidance Document on Terrestrial Ecotoxicology for the risk assessment of plant protection products. Currently, the assumption is made for non-target arthropods that the peak concentration of pesticide residues after the last application is determining the effect level and thus risk in the first-tier as well as in the higher-tier assessment (except for some assessments based on semi-field or field studies). Calculation of risk quotients for multiple applications of products is then based on tests with one single application of the product, and the derived effect levels are compared with estimated final residues on soil or leaf surfaces after the last application. In non-target terrestrial plant risk assessment, multiple applications are currently not considered in written guidance, although some authorities do apply factors to account for an accumulation of residues following multiple product applications. There is also a recommendation in OECD test guideline for evaluating toxicity to plants that multiple applications should be considered when limit tests are conducted, but this would typically be achieved by cumulating a sequence of intended product application rates into one single test application rate. In this presentation we look into the current approach for assessing multiple applications in the risk assessment for plant protection products. As mentioned, this is normally done by applying factors that account for increased exposure levels in the target compartment, whereupon possible consequences of multiple impacts on the affected non-target organisms are not yet considered. However, there are situations where it appears more plausible that an overall level of effects in a multiple-exposure scenario is caused by accumulation of effect levels rather than by accumulation of exposure levels. We demonstrate a simple approach for estimating cumulated effect levels that makes use of dose-response information from toxicity tests. The applicability of such ‘multiple effect factors’ as compared to exposure-oriented ‘multiple application factors’ is discussed for different scenarios, considering ecological and physiological parameters of potentially affected groups.

MO286

Mixture effects of herbicides to aquatic macrophytes: sensitivity and recovery potential under environmentally realistic conditions

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Duckweed species, even though used excessively in risk assessment of plant protection products (PPPs), are not considered to be very sensitive to certain substances with specific mode of action, such as auxin simulators. Based on low toxicity to *Lemna* sp., many PPPs containing active substances such as 2,4 D, dicamba etc. are considered to be products which pose minimum risk to aquatic ecosystems. However, our previous studies have shown not only that 2,4 D is toxic to other aquatic macrophytes (e.g. *Myriophyllum* species), but that if recovery potential after exposure was taken into consideration, 2,4 D had significant, but delayed effect on *Lemna minor*, too. Worldwide, 2,4 D is one of the most heavily used herbicide, either as a single active substance, or in products containing two or three active substances, including atrazine in USA. Still, toxicity data for commercial herbicide mixtures, not to mention environmentally relevant coctails under realistic environmental conditions, are rather scarse, even for standard species such as *Lemna sp*, let alone other aquatic plants. Than again, it is a well known fact that toxicity in aquatic ecosystems is usually associated with mixtures of various compounds, under varying environmental conditions. Therefore it is essential that potential risk of multi-component mixtures to the natural populations, communities and ecosystem integrity is adequately assessed. The aims of this study were to a) assess whether binary mixture containing 2,4 D (close to IC_{10}) and atrazine (in individual $IC_{10,\ 25,\ 50}$ concentrations) have significant combined, presumably potentiating, effects on *Lemna minor* with special focus on recovery patterns after exposure to mixture vs. individual substances; b) to contribute to largely non-existing data on PPPs mixture toxicity to *M. aquaticum* and to evaluate suitability of newly proposed water-sediment test system with *Myriophyllum sp*. for applications other than routine regulatory – driven single substance testing and c) to focus on the potentially unexpected interaction between selected herbicides (as single substances as well as binary mixture) and dissolved organic carbon (DOC), an important environmental factor which can influence both bioavailability and toxicity of xenobiotics. This study was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia via Grant No. 172028.

MO287

Multiple pulse exposure studies with Algae, Lemna sp., and Myriophyllum sp. – technical considerations

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Plant production products are, due to their purpose, toxic to different trophic levels in the environment. Herbicides are, in most cases, very toxic to all kinds of plants. Therefore a refined risk assessment is necessary to enable both the use of these herbicides in agriculture and the protection of the environment. One possibility to enable a refined risk assessment could be the performance of higher tier studies such as pulse dose studies. Arising needs of those pulse studies with Algae, *Lemna sp*., and *Myriophyllum sp*. lead to special considerations regarding the technical performance. The changes from test concentrations to pure test water or vice versa can be challenging depending on the test species. Frequency of parameter assessment (e.g., measurement of algal biomass, counting *Lemna sp*. fronds, measuring *Myriophyllum sp* shoot length), frequency of test media change, frequency of analytical verification of test concentrations and the removal of test substance during the recovery phases need a careful planning phase with respect to the purpose of the test. The impact on the growth of the test organisms caused by the frequent manipulations of the growth conditions, needs to be taken into account during evaluation of the results. For instance, *Myriophyllum sp*. plants are highly impacted by test medium change, since the plant morphology is adapted to the submerse growth. Removing the medium leads to an exposure to the air and refilling the test vessel with new test medium causes an air buffer around the plant. The significance of the endpoints, which could be assessed, needs to be rethought. Considering the rough changes in growth conditions, only integrative parameters like growth rate give valuable endpoints. This presentation will give insights into the implementation of a multiple pulse exposure study from a technical point of view.

MO288

Pesticide Half-Lives in Plants for Risk and Impact Assessment

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Pesticide risk and impact assessment models rely on data describing dissipation from plants. Dissipation is a key mechanism for assessing pesticide distribution and the magnitude of residues in harvest. To reduce uncertainty linked to pesticide dissipation in plants, a consistent approach for characterizing dissipation kinetics based on available experimental data is required.\nWe characterize 4442 measured pesticide dissipation half-lives and determine the influence of temperature from a subset with reported study condition temperatures. We provide recommended geometric means of dissipation half-lives at 20°C and 95% confidence intervals for 333 reported pesticides, and use multiple imputations for substituting missing temperature data. Finally, we propose a regression-based model to predict dissipation half-lives for pesticides as a function of temperature, substance chemical class, selected substance properties and plant characteristics.\nBased on fitting experimental pesticide dissipation data in plants with reported temperatures, we obtain a reaction activation energy E_a=14.25 kJ/mol, a temperature coefficient Q_{10}=1.22, and an Arrhenius constant A=1.02 day^{-1} to correct dissipation from plants for the influence of temperature.\nCalculated recommended dissipation half-lives range from 0.2 days for pyrethrins to 31 days for dalapon with 95% of all half-lives falling in the range between 1 and 18 days. In our final predictive model, substance class, plant species, cold storage conditions, temperature, substance molecular weight, octanol/water partition coefficient and saturation vapor pressure are taken into account as predictor variables yielding an R^2 of 0.49. Analyzing the relation between dissipation and degradation yields deviations of less than a factor 2 for 90% of our data, thus the recommendation to perform a sensitivity analysis by varying the applied half-life by this additional factor 2.\nConsidering the relatively small deviation between dissipation and degradation half-lives, high variability between substances and plants, and data availability, our recommended reference half-lives along with the final predictive model constitute a first step towards reducing uncertainty in risk and impact assessment models with respect to pesticide degradation in plants.

MO289

Towards a better understanding of foliar wash-off: Use of molecular dynamics simulations to elucidate surface interactions at the nanoscale

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Foliar wash-off is the removal of substances from plant leaves by rain. This process

is implemented in environmental fate models which are used in the context of the registration of plant protection products. In general, these models are carefully parameterized using experimental data collected from macroscopic systems, such as soil samples or field studies, and are relied upon to make predictions based on representative worst case scenarios. To gain fundamental insight into foliar wash-off at this scale, one also requires a better understanding of the relevant system interactions contributing to this process *at the nanoscale*. This is, however, a non-trivial multiscale problem. To study the interplay of interactions involved, molecular dynamics simulations are employed to study the liquid-vapor, solid-air, and solid-liquid interfaces, where the leaf’s waxy surface is approximated by a rough hydrophobic landscape.

MO290

Bioremediation of polluted sediments by rooted aquatic macrophyte Typha domingensis.

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Sediments are a compartment of aquatic ecosystems that are often highly contaminated by chemicals that have been introduced into de water body. They are recognized as the major sink for different kinds of contaminant including persistent and hazardous substance. Among persistent compounds heavy metals are the most common elements which can lead to accumulation, transport and ecotoxicity to living organisms. Sediments are a compartment where important biochemical transformations and biological process take place. Concern of this problem had led to the development of alternative technologies for the removal of metal from contaminated sediments. Rooted aquatic plants can be used for this task. Te aim of this work was explore the removal capacity of Chromium, Zinc, Lead and Nickel from contaminated sediment by the rooted macrophyte *T. domingensis* performing the assessment of the decrease in whole ecotoxicity of sediments by a panel of bioassays with primary producers. The sediment was collected from a stream that flows across an industrial area receiving wastewater discharge mainly heavy metals and persistent organic susbstances. Physic-chemical parameters as well as metal determinations were made. Chromium, Zinc, Lead and Nickel were the metal in major concentration. Prior to removal experiences, initial assessment of whole ecotoxicity of sediments were made with rooted submerged *Miriophyllum elatinoides* (native specie) according to general recommendations of Feiler *et al* (2004; 2012). At the same time, eluate of sediment was prepared in order to assess initial ecotoxicity by performing standard assays with algae *P. subcapitata*, *S. quadricauda* and the floating macrophyte *Lemna gibba*. Plants of different ages of *T. domingensis* were incubated in whole sediment during a month in plastic containers in an outdoor system. At the end of the removal period whole sediment was removed and eluate was prepared. Same battery of primary producer mentioned above was performed both whole sediment and eluate. The results showed that *T. domingensis* can be successfully used for heavy metal removal as a decrease of whole sediment ecotoxicity was achieved. Adsorption or bioacumalation process involved in this removal was proposed. The battery of primary producer applied was suitable in the detection and evidence of this decrease, specially the value of the rooted *M. elatinoides* as supplement to existing test battery in order to improve the assessment of sediment toxicity.

MO291

Genotoxicity of pesticides on green algae: linking cellular biomarkers to dynamic populations.

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Aquatic environments of the pampasic region ofArgentinareceive chemicals from agricultural activity due to an increase in a glyphosate tolerant transgenic variety of soybean crops. Non target organisms, as aquatic primary producers are severely affected by this agricultural contamination. In the present study genotoxicity effects as well as growth patterns evaluations of commercial formulations of insecticide Chlorpyrifos and fungicide Tebuconazole on green algae species was done. Recovery studies were also done in order to differentiate algistatic from algicidal effects. Citotoxicity evaluations of pesticides on algal cells were done prior to genotoxic assays through fluorometric and autofluorescence determinations. Genotoxic damage was evaluated by Single Cell Gel Electrophoresis Assay (SCGEA). Growth inhibition toxicity test of 96 hours were done with two species of green algae. After a contact period to pesticides, a recorded of cell density during ten days were done in order to evaluate recovery after pesticide exposure. Growth rates and growth models were obtained for each exposure and recovery to pesticides. Genotoxicity studies were performed by exposing microalgae during its exponencial phase to selected concentrations of pesticides derived from growth test, for 24 hours. At the same time exposition to hydrogen peroxide, a model genotoxicant was made. Following exposure, cell suspension was concentrated by centrifugation and resuspended in PBS. Cell viability was assessed.The comet parameter percentage of tail DNA was calculated using image analysis. Growth rates as well as growth patterns of both microalgae were altered at the end of exposed period to both pesticides. Chlorpiryphos exerted algicidal effect while Tebuconazole was algistatic. Growth patters were modified in the recovery evaluations, as a decrease of biomass significant different for control was obtained. Pesticides at subletals concentrations caused genotoxic effects on microalgae cells, allowing the detection of early effects that were evident at population level and at recovery capacity. LOEC obtained from growth inhibition tests of 96 hours were the same where genotoxic effects were detected after 24 hours of exposure to pesticides. In this way this biomarker at cellular level resulted of great predictable capacity of effects that not only are worse in time but highlighting alterations on ecological attributes of higher level of organization as population level.

MO292

Detection of toxic substances using biosensor

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Environmental pollution currently reaches up such level that systematic selective control of polluting compounds is almost not possible. On contrary, in accordance with sustainable development, it is necessary to ensure sufficient quality of the environment for future generations. This is associated with regular quality control of components of the environment, which is implemented currently by classical analytical methods and toxicity tests in laboratory conditions. A comprehensive evaluation of environmental pollution and the impact of pollution on living organisms, however, can be performed only by accredited, authorized or otherwise certified methods. The above is the cause of large time and financial demands to assess whether environment on a particular locality may pose a risk to living organisms or not. The solution may be the application of biosensors that can greatly simplify the measurement. They significantly reduce the costs and time necessary for obtaining information of the same quality as classical measurement. The advantage of biological tests is that they allow the detection of the total effect of chemicals on the environment and living organisms. They are able to measure even the most harmful substances whose classical analysis is not methodologically managed yet, but their common feature is toxicity for sensitive organism. Very suitable organisms due to their sensitivity to environmental pollution are algae. Therefore the new device based on the principle of the biosensor indicating the environmental burden by pollutants was developed. The principle consists of measurement of life cycle of algae. The life cycle is monitored by oxygen production after algae illumination. The device consists of bioreactor with algae suspension (5 ml), stirrer, oxygen electrode and minithermostat to stabilize temperature during the experiment. Oxygen concentration is measured with precision 0,02 %. 24 hours signal stability is 0,1 %. Response time is 2-3 s. These parameters enable to detect the change of oxygen production induced by traces of toxic compounds with limit of detection less than 1 μM. Experiments were performed on green algae *Scenedesmus quadricauda* a *Pseudokirchneriella subcapitata*. Measurements were performed using both selected chemical compounds ($K_2Cr_2O_7$, 3,5-dichlorfenol, $AgNO_3$) and natural environmental samples of runoff waters taken next to highway with high traffic intensity. The results were compared with ISO 8692.

MO293

Effects of ultraviolet radiation on chemical toxicity in the green alga Chlamydomonas reinhardtii: the role of the chemical mode of action

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Environmental stressors, such as ultraviolet radiation (UVR) can interact with the effects of chemicals pollutants, causing multiple stressor effects that can be higher (synergism) or lower (antagonism) than expected based on the effects of the individual stressors. The corresponding mechanisms are not well investigated. It has been suggested that effect interactions depend on the similarity of the mode of action (MoA) of the environmental and chemical stressor. To test this hypothesis, we exposed the model green algae *Chlamydomonas reinhardtii* to increasing concentrations of cadmium and the herbicides paraquat, diuron (DCMU) and S-metolachlor, with and without UVR of a fixed intensity. Considered endpoints were growth and photosynthesis. Single and combined stressor effects were examined based on the assumption that effects of the individual stressors are independent (independent action [IA]), which was assumed for chemicals causing effects through mechanism dissimilar to those of UVR. Interaction of effects were examined by comparison of concentration response curves for all chemical-UVR and chemical+UVR treatments and by comparison of measured combined effects with IA-model predictions of combined effects. Effects on photosynthesis of paraquat, DCMU and Cd, assumed to act similar to UVR, were found to interact with the effects of UVR. Synergistic effects of paraquat and Cd in combination with UVR assessed particularly at higher concentrations, may be explained by the ability of these different stressors to increase the generation of toxic reactive oxygen species in the chloroplast, thus causing aggravated effects when acting together.

Antagonistic effects of UVR and DCMU, assessed also at low concentrations, may have resulted from a UVR induced modification of the herbicides target site, reducing its affinity to the herbicide. Effects of S-metolachlor, assumed to act dissimilar to UVR, were independent from UVR effects on photosynthesis, but not on growth. Synergistic effects on growth at higher concentrations may be explained by effects of S-metolachlor on the stability of the algal cell membranes, enhancing their sensitivity to UVR. For chemicals for which synergistic effects were observed, effects in combination with UVR were generally well predicted by the IA-model at low concentrations, but underestimated by a factor of less than 2 at higher concentrations.

MO294

Estimation of toxicity and genotoxicity of bottom sediments of the Yenisei River: Elodea canadensis vs. Allium-test

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The Yenisei River has been subjected to radioactive contamination due to the operation of Mining-and-Chemical Combine (Rosatom) (MCC) producing weapon-grade plutonium for more than fifty years (1958-2010). As a result, high activities of long-living artificial radionuclides (^{137}Cs, ^{238,239,240,241}Pu, ^{241}Am) were deposited in sediments of the river. Bottom sediments (BS) of the Yenisei River downstream the Krasnoyarsk city are also polluted with heavy metals (HM) because of industrial discharges and from water catchment area. The purpose of this research was to estimate the toxicity and genotoxicity of BS of the Yenisei River with *Allium*-test and new developed sediment-contact test using submersed macrophyte *Elodea canadenis* (elodea). Chromosome abnormalities in roots of elodea, sampled in the Yenisei River were estimated as well. For ecotoxicological experiments BSs were sampled in the Yenisei River upstream and downstream of the MCC effluents. Samples of BS contained natural isotope ^{40}K (240-330 Bq/kg, fresh mass) and artificial radionuclides ^{60}Co (max. 70 Bq/kg), ^{137}Cs (0.8-1400 Bq/kg), ^{152,154}Eu (max. 220 Bq/kg), ^{241}Am (max. 40 Bq/kg). Apical shoots of elodea and onion bulbs were planted in sediments (10 shoots or bulbs per sediment subsample in three replicates). Endpoints of elodea shoot and root growth and onion root growth were used as toxicity indicators; the rate of cells with chromosome abnormalities in apices of elodea and onion roots (%) - as a genotoxicity indicator. Sensitivity (% of inhibition related to control) of elodea endpoints to the quality of BS increased in the row: shoot length (22 %) < root length (42 %) < root number (44 %). Shoot length endpoints negatively correlated ($p < 0.05$) with radionuclide concentration in samples of BS. Positive correlation of chromosome abnormalities rate ($p < 0.05$) with radionuclide concentration in samples of BS was revealed for elodea and onion as well as for natural population of elodea from the Yenisei although the mechanism of this effect is unclear. Spectrum of chromosome abnormalities was bigger in roots of onion. Correlation of shoot and root growth with concentration of HMs in BS was not revealed. Sensitivity of elodea and onion endpoints to the concentration of radionuclides and HMs in BS was compared. We can conclude that *E.canadensis* can be recommended as sensitive species for testing of toxicity and genotoxicity of BS in laboratory assays and *in situ*.

Pollinator risk assessment: past, present and future (P)

MO295

Impact of legislation about pesticides in the conservation of stingless bees in Brazil

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For the registration of a pesticide in Brazil is required data for their toxicity to bees. However, the data presented are obtained by testing the species Apis mellifera which is not naturally found in the country. In fact, the tests do not even reflect toxicity to the existing hybrid in Brazil, since they are made to European subspecies. Thus, the aim of this study was to conduct an analysis concerning differences found between the LD_{50} and LC_{50} of fipronil in established product registration in relation to those established for the Africanized hybrid and two species of brazilian bees *Scaptotrigona postica* and *Melipona scutellaris*. Data were obtained from the methodology established by the OECD for testing water contamination by contact and oral (OECD guidelines 213 and 214) at the Center for the Study of Social Insects , Universidade Estadual Paulista , Rio Claro campus . The LD_{50} for bee Apis mellifera varies from 4 to 6.2 ng/bee and no data is avaliable to LC_{50} . For Africanized bee and the LD_{50} value was set at 1.06 n/bee LC_{50} and 1.27 ng/uL diet . The results for the stingless bee *S. postica* were 0.54 ng / bee and 0.27 ng/uL diet and *M. scutellaris* 0.41 ng/bee 0.011 ng/uL as LD_{50} and LC_{50}. The data show that the values used for registration are not protective for stingless bees, which are much more sensitive to these molecules. The use of products based on the doses established for Apis mellifera can endanger the colonies existing in remnants of the native areas that can be exposed when visiting the crop search for food, while providing the environmental service of pollination colonies. The use of non-native especies might compromise the conservation of diversity in the country and impact on agricultural production for the loss of essential pollinators to endemic species and for several crops of economic importance. For better conservation of native species is vital that include studies on the toxicity of molecules to native bees. It is also important evaluating routes of exposure of these bees in agricultural crops, since they have biology and behavior very different different from that observed in *A. mellifera*.

MO296

New methodology for proboscis extension reflex test for stingless bees

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Bees are non-target arthropods and are also excellent pollinators contributing to the biodiversity of plants and are additionally economically relevant. The relevance is given due to pollinating activity on plants especially crop plants. Besides to direct mortality induced by pesticides to pollinators, sublethal effects must be considered for a analysis of pesticide impact. An increasing number of articles about this subject have been published in the last 10 years. There was even a special science issue 2013 on the subjects. But many papers are restricted to behavioral effects in some species, mainly honeybees (*Apis mellifera*). One test method for sublethal effects on bees is the proboscis extension reflex (PER). The PER is used to assess the sucrose responsiveness under laboratory conditions. Normally the test is applied to restrained individuals The PER bioassay was used in several publications from different authors to assess behavioral effects of pesticides. In a number of papers difficulties to use this method with non-Apis bees were observed. The conclusion was that the basic technique used for eliciting the PER in honeybees cannot be applied to other bee species. The aim of this study was to evaluate the sucrose responsiveness of two stingless bees, *Melipona scutellaris* and *Scaptotrigona postica* using two different protocols. The first protocol included the traditional method where forager bees remained restrained. In the alternative protocol bees could move freely. In both cases, the bees were inserted into modified plastic centrifuge tubes with the tip cut out. Prior, the bees were anesthetized (freezing) and insert into the tubes. After a starvation period an increasing concentration of sucrose-water solution (w/v) was offered. Between the solutions, water was offered. With the traditional method, the sucrose responsiveness was observed only for *M. scutellaris* bees (12.5% of tested bees) in just one sucrose concentration (75%). Using the methodology with free movement, both species showed sucrose responsiveness in all tested concentrations (25%, 50% and 75%). The number of *M. scutellaris* bees with sucrose response ranged from 53.7% to 76.2 % depending on the sucrose concentration and the number of *S. postica* ranged from 54% to 79%. These results showed that using the alternative methodology with free movements the sucrose responsiveness can be assessment in some non-Apis bees. Such an approach can result in a larger base of data to test the sublethal effects on individual bees.

MO297

Make a BeeCision – Is insecticidal activity of a PPP a criterion to trigger laboratory studies with non-Apis bees?

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Over the last six years, the effects of plant protection products (PPP) on pollinators such as honey bees have come increasingly to the attention of both scientists and the general public. In 2013, under the new EU Regulation 1107/2009, the European Food Safety Agency (EFSA) published a new guidance document on risk assessment for pollinators. In addition to assessments on honey bees, the new guidance requires acute and chronic risk assessments for adult bumble bees and solitary bees as well as chronic risk assessment of bee larvae development. Because validated test guidelines for non-*Apis* bees will not be available in the near future, in the absence of other data, risk assessments for these species are based on honey bee toxicity endpoints. However, non-*Apis* risk assessments based on honey bee data sets are very conservative. PPPs therefore frequently fail the initial screening step and higher tier testing is automatically triggered. In accordance with the new EFSA guidance document, we conducted risk assessments on honey bees, bumble bees and solitary bees on numerous compounds including 20 herbicides approved for use in Europe. The non-*Apis* risk assessments were based on honey bee toxicity endpoints obtained from data sets available to the public (e.g. EFSA or the European Commission). All 20 herbicides failed the initial screening step for bumble bees and solitary bees. Moreover, refinement with actual residue and sugar content data will probably not lead to a better evaluation. Nevertheless, risk assessments conducted on non-target arthropods (*Aphidius* and *Typhlodromus*) suggested that many of the herbicides have little or no insecticidal activity. In particular, risk assessments for 13 of the herbicides suggested these compounds posed no risk to either the standard arthropod species or honey bees indicating a low risk to all insects including pollinators. In order to assess the risk posed by non-insecticidal PPPs to bumble and solitary bees more realistically and bridge the time until suitable testing guidelines are available, we propose the use of the

'BeeCision' tool. This tool reinstitutes the 'insecticidal activity' approach originally suggested in the draft EFSA Guidance Document and triggers further tests on non-*Apis* species only when potential insecticidal activity is clearly demonstrated. The current presentation evaluates the benefits of this approach and discusses its potential use as an aid to assessing the risk posed by PPPs to bees.

MO298

The challenge of assessing the risk of pesticides to non-Apis bees and the outcome of the EFSA approach

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The deterioration of wild bee fauna is connected with habitat fragmentation, the growing distances between nesting sites and foraging fields as well as competition with cultivated bees in agricultural habitats. Growing concerns over pollinator declines demands a risk assessment procedure meeting the protection goals of all bee organisms. In consequence, not only honeybees but also bumblebees and solitary bees are assessed in the new EFSA risk assessment procedure for registration of plant protection products. First-tier generic values were used as a measure of the sensitivity of the different risk assessment schemes in the EFSA guidance document. A risk assessment sensitivity quotient (SQ) is derived based on shortcut values, exposure factors and trigger values for all foreseen exposure scenarios. The quotient allows direct sensitivity comparison among risk assessment schemes of the three different bee organisms, honeybees, bumblebees and solitary bees. The evaluation of SQ values reveals that the most sensitive risk assessment outcomes are nearly always to be expected for bumblebees or solitary bees. The greatest difficulties will most likely come up in the exposure routes via weeds in the field and via plants in the field margin where the major differences to honeybees' risk assessment were identified. Provided that toxicity data is available the SQ values of bumblebees and solitary bees are considerably higher indicating higher sensitivity. The difficulties even increase in a risk assessment only based on honeybee toxicity data which is mostly the case. The proposed EFSA exposure assessment gives a list with information about bee attractiveness of common crops in Europe. With respect to bumblebees and solitary bees, only the crops are indicated which were observed to be visited by those bees, and lacks of data telling whether crops are non-attractive to wild bees. It must be reckoned that at least one of the wild bee species would feed on nectar and pollen from flowers as soon as the crop provides any that is convenient to bees. Under these circumstances, the risk assessment will frequently suggest bumblebees and solitary bees foraging in treated crops even though scientific literature gives evidence that cultivated honeybees are more abundant in agricultural landscapes.

MO299

Landscape-level exposure assessment for bees: spatially-explicit modelling approach to assess the influence of foraging behaviour on in-hive exposition

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Within the recent EFSA guidance document on the risk assessment of plant protection products on bees (EFSA, 2013), the risk assessment for the consumption of nectar and pollen in the hive is based on the conservative approach that bees forage exclusively on one type of plant. The guidance gives options to replace this conservative with a more realistic approach considering foraging on all attractive plants in the foraging area at the landscape level. In the guidance document a simple model approach is presented that assumes that the fraction of honey bees foraging in each field within a certain maximum foraging radius, is proportional to the product of attractiveness (e.g. defined by nectar yield) and area of each field. In this study, we use a stepwise approach to further refine this modelling approach, by taking into account distance effects, by the quantification of foraging costs and yield in terms of energy, and by considering depletion effects in the fields. We developed spatially-explicit model versions based on national geographical information system data sets. Model runs of the different refinement steps of the model were performed using data on crop cover per individual field (originating from the land use database of the Netherlands). For the single refinement steps, dilution factors were calculated for all possible bee-hive locations in the landscape, thus quantifying the dilution of the concentration of a pesticide that is theoretically found immediately after a spray event in nectar brought into a hive situated next to a treated field. Calculations for the initial model and the first refinement step (taking into account distance from field to beehive), indicate that dilution is consistently less when taking into account distance effects. Further simulation results will show the impact of increasing the realism of the foraging behaviour of bees on the dilution factors. The final aim of this work is to arrive at a generic and robust, but realistic description of the influence of the foraging behaviour and of the existing landscape structures – including off-crop habitats – in the Netherlands on the pesticide residue levels in nectar returned to the hive. It is the intention then to use this model in the risk assessment procedures of plant protection products for honey bees.

MO300

Evaluating EFSA honeybee protection goals using the BEEHAVE model

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EFSA has recently issued new guidance documents for risk assessment of plant protection products to honeybees. The protection goals were operationally defined as colony size (number of bees) and effects should not exceed a 7% reduction [1]. Additionally, mortality of foragers should not be increased by more than a factor of 1.5 for 6 days or a factor of 2 for 3 days or a factor of 3 for 2 days. These limits for forager losses were set based on a very simple honeybee model [2,3] Here, we use a more realistic honeybee model, BEEHAVE, [4] to explore the potential impact at the colony level. BEEHAVE combines in-hive processes with landscape level forage availability via a foraging module. We developed scenarios based on the "Forager mortality after pesticide treatment" from [4]. We explored the impact at the colony level of different timings and magnitudes of forager losses and overall reduction in colony size. Preliminary findings show that the EFSA protection goals are clearly very protective. The protection goals were derived using a very simple honeybee model that does not include many of the factors and feedback mechanisms, which enable honeybees to succeed in a fluctuating environment (e.g. honey stores were not included). Repeating some of the EFSA simulations with this more realistic model shows that inclusion of these factors and feedback mechanisms gives a much lower estimate of risk. Our simulations also showed that there is a strong temporal component to risk so timing of exposure has a large impact. [1] EFSA 2013. Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 2013;11(7):3295. [2] Khoury, Myerscough, Barron 2011. A quantitative model of honey bee colony population dynamics. PLoS ONE 6, e18491 [3] Becher, Osborne, Thorbek, Kennedy, Grimm. 2013. Towards a systems approach for understanding honeybee decline: a stocktaking and synthesis of existing models. Journal of Applied Ecology. DOI: 10.1111/1365-2664.12112 [4] Becher et al. BEEHAVE: A systems model of honey bee colony dynamics and foraging to explore multifactorial causes of colony failure. *Submitted*.

MO301

Nest for cavity-nesting solitary bees, particularly Osmia species, in ecotoxicology studies.

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Conventional nest systems used for research or commercial pollination with solitary cavity-nesting bees, in particular *Osmia lignaria*, *Osmia cornuta* and *Osmia bicornis*, have limitations in ecotoxicology testing. The authors propose a novel nest which enables a high level of observation and data collection to be made quickly and easily, while minimising disturbance of either the nesting mothers or the juvenile bees within the nest. \nThe nest comprises a series of stacked layers, each layer providing an array of cavities of size and material which are acceptable to nest-seeking females. Each layer is made up of a sandwich consisting of a rigid base, a light-weight floor, a machined nest laminate, a clear cover and a rigid cover. These elements are clipped or screwed or otherwise fixed together; a stack of nest layers is deployed in a housing which is constructed so that each layer may be slid out or otherwise removed without disturbing the adjacent layers.\nEach nest may be examined at any time by sliding it from the housing and removing the rigid cover. The contents - food provisions, eggs, larvae, prepupae, cocoons and parasites - may be seen through the clear cover. Samples may be removed for examination and analysis by cutting through the clear cover, then returned if required. A graticule printed on the clear cover enables the identification of each item in each layer.\nThe dimensions of the nest layer are such that the nest and contents can be x-rayed without disturbing the individual bees, by removing the rigid base.\nThe nest simplifies observations which previously were difficult or impossible, and enables the development of rigorous, reliable and objective ecotoxicology protocols.

MO302

There's no space like home: Reduction in control mortality by mimicking bee space in the test vessel design for acute Honeybee Apis mellifera toxicity testing

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Following the OECD Guidelines 213 and 214 Acute Oral and Contact Toxicity Tests, control mortality of two differing test vessel designs were compared for overall percentage mortality and honeybee behaviour. The vessel types consisted of a cylindrical steel cage vs. a clear plastic design. Honeybees from the same supplier hives were used to determine if a change in vessel design would impact upon a number of factors, namely: response to anaesthesia, behaviour, feeding and a reduction in control mortality. In total > 1000 control bees were "exposed" using the steel cage and clear plastic designs for comparison. Perhaps more importantly, a better accuracy for delivery of anaesthesia was devised, dramatically reducing the overall time required for the honeybees to respond to anaesthetic. Additionally, changes in the makeup of the sucrose feeders was investigated and natural honeybee hive behaviour, such as the "wagging dance" and arrangement in

symmetrical patterns were observed indicating that the honeybees favoured one test design over the other.

MO303
A short survey of pollinators on cover crops in southern Germany 2013
S. Knaebe, EAS EcoChem GmbH / Ecotox Field; P. Mack, EAS EcoChem GmbH
The new risk assessment guidance for plant protection products in bees was published last year. Due to the clear lack of data on several subjects addressed in this guidance no conclusion for the risk assessment could be reached. One point that has been addressed is the exposure assessments of solids, focusing on the drilling in spring. In Europe, however, autumn drilling is an important time for winter crops, such as oil seed rape and cereals. In central Europe winter cereals are drilled up November. Under normal conditions there would be very few flowering plants. However, common agricultural policy requires from the farmer to plant a cover crop on the field that are harvested before the middle of September. Two common cultures in southern Germany are *Phacelia tanacetifolia* and *Brassica nigra* (mustard). If the weather is mild both cultures can flower before frost starts and are visited by a wide variety of insects. The flowering cover crops are adjacent to the fields seeded with treated seeds. 2013 was a mild autumn with very warm days increasing the likelihood of visiting pollinators. To find out more about the abundance and diversity in the flowering cover crops a small pollinator survey was run in two flowering fields of *P. tanacetifolia* and two fields of *B. nigra*. The survey was done be establishing three bee bowls in the center of each field and three bee bowls at the edge of each field. Bee bowls are small colored plastic bowls or cups that are filled with water and detergent. Pollinators are attracted to these colors, fly into the water and drown. One of the bowls is white; one is yellow and the third one blue. Bee bowls were sampled over 4 days with warm weather where pollinator flight was likely. The main pollinators sampled were honey bees but other arthropod families were sampled, like for instance specimens belonging to Lepidoptera or Diptera. The short survey proved that cover crops can be attractive for pollinators if the conditions for flight are favorable, leading to a possible exposure to PPP in autumn.

MO304
Evaluation of Honeybee Acute Toxicity of plant extracts, Neem, Sophora and Derris
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This study was performed to evaluate the risk of the environment-friendly agricultural materials formulated with Neem, Sophora and Derris extracts to Honeybees(Apis mellifera L.) through the acute contact toxicity test and acute oral toxicity test. As a result of acute contact toxicity test to honeybees, 48h-LD50 of neem and derris extracts were more than 100 ug/bee while 48h-LD50 of sophora extracts were 1.7 ug/bee. In case of acute oral toxicity test, 48h-LD50 of neem and derris extracts were more than 100 ug/bee while 48h-LD50 of sophora extracts were 1.7 and 0.3 ug/bee. In conclusion, it is evaluated that neem and derris extracts are practically nontoxic while sophora extracts are highly toxic to honeybees.

REACH after the second registration deadline: Environmental challenges (P)

MO305
A discussion of the sufficiency of aquatic hazard information for environmental risk assessment
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In the current regulatory arena, there exists a large amount of ecotoxicity data for aquatic organisms. There exists no clear consensus concerning whether available information on the aquatic hazard of substances is sufficient for determining whether or not an environmental risk assessment may be required for all compartments. This question gained relevance in the REACH context: under REACH, the requirement of a risk assessment is triggered by classification. As the current system of environmental classification only covers aquatic hazards, this is leading to cautionary approaches as to when and how environmental risk assessments are undertaken. For example, the discussion on the scope of Exposure Assessment under REACH is the subject of thorough debates concerning whether the existing classification for aquatic hazards captures the risks to organisms in the soil and sediment compartments. This poster will summarise the findings of an ECETOC Task Force on the matter. A database of existing aquatic, soil and sediment data for like substances will be analysed for correlation in sensitivity across compartments. A review of the current C&L system, and to what extent it is protective for risks in the water, soil and sediment compartments will be discussed, as will a review of available strategies for obtaining relevant environmental compartment hazard information. Finally, the discussion will cover cases where the existing aquatic data is sufficient to capture hazards/risks for all the compartments, as well as cases where aquatic information may be insufficient to enable a conclusion on the risk to all environmental compartments. Some proposals on resolution of these insufficiencies will be made.

MO306
Characterising the toxicology and ecotoxicology of industrial chemicals in Europe
M. Gustavsson, Biological and Environmental Sciences; T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences
Industrial chemicals have to be registered in Europe under the REACH Regulation (EC) 1907/2006 if they are put on the European market at more than 1 ton/year. Existing chemicals are phased stepwise into REACH, in May 2013 the deadline for the registration of chemicals in the 100-1000 tons/year band passed. The publicly available database at the European Chemicals Agency (ECHA) lists all currently registered chemicals and currently contains the basic hazard descriptors for 10655 unique substances from 41973 dossiers (Nov. 2013), in the form of PNECs (Predicted No Effect Concentrations) and DNELs (Derived No Effect Levels). In order to characterize the toxicological and ecotoxicological hazard of chemicals in Europe a distribution analysis of these data was performed. They will be presented with a view to answer the following questions: (i) what is the maximum, minimum and average (eco)toxicity of an industrial chemical in Europe?; (ii) is there a difference between the (eco)toxicities of compounds from different production volumes (tonnage bands)?; (iii) do the data allow to identify differences in the sensitivities of the different environmental compartments? (iv) is there a correlation between basic physico-chemcial characteristics (molecular weight, vapour pressure, lipophilicity, etc) and (eco)toxicity? The results from the analysis of industrial chemicals will be contrasted with similar (eco)toxicity distributions of pesticides, priority pollutants from the water framework directive and marine priority pollutants.

MO307
Shortcomings in ecotoxicity data to provide a testset for an ECETOC project
P. Thomas, CEHTRA SAS; M. Galay Burgos, ECETOC / Environmental Sciences Manager
Abstract An ECETOC Task Force was set up to validate a hypothesis that aquatic toxicity for narcotic substances can be accurately predicted by their aqueous solubility concentration. 2000 datapoints were located, the majority from the ECHA disseminated database. Only studies with a Klimisch score of 1 or 2 were used for assessment. The robust summaries of these studies were further assessed by the Task Force. A large number of studies were found not to meet basic obligations necessary to fulfill validity requirements (e.g. duration of the experiment), a further significant group of studies failed to put in place the appropriate methodological design in order to provide assurance that the test substance concentration was maintained over the study period. Several studies reported toxicity greater than the water solubility limit of the test substance. Overall, nearly 50% of the located data were not considered fit for purpose despite being classified as valid in the ECHA database. The methods used and results are reviewed. **Keywords** : ecotoxicity, dataset, study validation

MO308
Dialog between environmental monitoring data and REACH
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Numerous organic chemicals are produced and used on a daily basis. REACH (Registration Evaluation Authorization and Restriction of Chemicals) should guarantee the safe use for the environment and human health in Europe. Industry is obliged to provide theoretical and experimental data on their imported and produced chemicals. These data are used by risk assessors to determine potential (environmental) risks and advice legislation. Currently, the assessment of environmental risks of chemicals uses amongst others theoretical and experimental sorption, persistence and toxicity data to calculate predicted environmental concentrations and no-effect concentrations. Within REACH there are no measured concentrations of chemicals in the environment used to routinely support the chemical safety assessment. The current study explores whether environmental monitoring data can be used to support screening or prioritization activities within REACH. This so-called “dialog” between monitoring data and legislation can support the various evaluation processes in REACH and can even be used to underpin subsequent regulation of chemicals where appropriate. A broad screening approach based on liquid chromatography coupled to high resolution mass spectrometry (i.e. Orbitrap) was performed on 5 wastewater treatment plant effluents and 2 surface water samples. The data files were screened using advanced software tools for the presence of 1076 unique accurate masses that corresponded to

1556 REACH registered substances. The matching revealed between 28 and 82 hits per sample. The number of confirmed elemental compositions was significantly higher in STP effluent samples than in the surface water samples, which was expected in view of the higher concentrations usually found in effluents. The non-target approach seems promising for screening purposes, It might be applied to support and potentially guide evaluation efforts of REACH dossiers. An advantage compared with using standard monitoring data, is that this approach might results in chemicals that are not part of classical monitoring data. However the uncertainty associated with the identity of the matching chemicals (*i.e.* accurate masses) makes it currently unsuitable for regulation purposes. However, when the identity of matching chemicals can be confirmed, which is a subject of a future research project, this approach can become a valuable evaluation tool to support regulation of chemicals.

Evolutionary, multigenerational and epigenetic effects of pollutants: providing scientific support to long-term ERA (P)

TU001
Chronic toxicity (multi-generational effect) of perfluorinated compounds (PFCs) on the daphnids
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Perfluorinated compounds (PFCs) such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanic acid (PFOA) are water-soluble, bioaccumulative, environmentally persistent pollutants, and their adverse effects on both human and wildlife have raised global concern. Because of their bioaccumulative and persistent property, long-term and chronic toxicity, especially, transgenerational effect of PFCs should be investigated. In the previous study, we evaluated trans-generational effect of PFOS on Japanese medaka (*Orizias latipes*), and observed toxicity increase in some endpoints (e.g. survival of larva) in the second generation. To confirm this toxicity increase is also observed in other aquatic species, we conducted multi-generational reproduction test using *Daphnia magna*. As a preliminary survey, toxicity of perfluorinated carboxylic acids with carbon chain C8 to C11 was tested using *Ceriodaphnia dubia* reproduction test. In the result, we found the toxicity increased as the number of carbon chain increased. Therefore, we selected perfluorodecanoic acid (PFDA, C10) as well as PFOS to be subjected to the multi-generation test. In the multi-generation test with *Daphnia magna*, the first generation of daphnids was pre-exposed to different concentrations of test chemical. The neonates from each test concentration continued to be exposed at the same concentration respectively as exposed groups. In addition, neonates from the control groups were also exposed to different test concentrations as un-exposed group. Trans-generational effects of chemicals were evaluated by comparing the number of neonates from exposed and unexposed group in the second generation. Pre-exposure to PFDA did not affect the number of neonates in the second-generation. However, the number of aborted eggs and dead neonates increased in the exposed group. Pre-exposure to PFOS also did not affect the number of neonates in the second-generation. Aborted eggs and dead neonates also observed at the higher concentration of PFOS; however, the number of them was not different between unexposed and exposed group. PFDA showed higher toxicity than PFOS; NOEC of PFDA was 5 mg/L whereas NOEC of PFOS was 12.5 mg/L. These concentrations were around 10^4-10^6 times higher than environmental detected concentration, indicating that the risk of PFOS and PFDA on the daphnids was considered negligible in the environment.

TU002
Transgenerational effects of Cd contamination in Zebrafish (Danio rerio)
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Cadmium (Cd) is a highly toxic heavy metal. This metal has been increasingly dispersed due to a wide range of industrial use of this metal. It has been stated by the International Agency for research on Cancer that ionic Cd causes genotoxicity effects in a variety of types of eukaryotic cells, including human cells and therefore Cd and its compounds was classified as a carcinogenic to humans and experimental animals. This metal is also known for inducing an increase in the reactive oxygen species (ROS) leading to DNA damage and apoptosis. It has been shown the effects of Cd in the DNA repair mechanisms and its effects as a teratogen being proposed as an agent leading to DNA hypomethilation. Due to high exposure to contaminant levels due to polluted effluents, fish (especially those inhabiting aquatic systems near urban areas) are among the vertebrates more exposed to pollutants. Contaminant resistance in this species may occur due to genetic adaptation or acclimation. Acclimatation due to physiological and epigenetic mechanisms may reduce the effects of toxic compounds, including Cd and Cd compounds. These are the first line of defense against environmental constrains, acclimation processes are not transmitted across generations and after the stressor is eliminated these mechanisms should disappear. However, acclimation may be passed through one generation by passing specific epigenetic markers which may confer resistance or deleterious effects as those refered above. Zebrafish (Danio rerio) is a well characterized vertebrate model with applications in many fields including biomedicine and biotechnology as well as ecotoxicogenomics. In this work, exposure of adult breeding pairs is undertaken in order to assess the effects of sub-lethal Cd levels in the progeny. Reproductive parameters such as clutch size, hatchability, fecundity and survival are assessed in order to evaluate its teratogen effects. Possible resistance in the progeny is assessed by fish embryo toxicity (FET) tests in the resulting generation in order to evaluate possible shifts in LC values to Cd toxicity. This study highlights the importance of considering the sub-lethal effects of environmental contaminants, not only to the exposed individuals but to upcoming generations which may be “weakened” by parental chemical exposure or, otherwise, inheriting acclimation processes conferring chemical resistance to chronic stresses which allow them to thrive.

TU003
Multi-generational effects of the feedlot contaminant 17 α-trenbolone on Daphnia carinata
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Anthropogenic chemicals can disrupt the endocrine systems of humans and wildlife. This has and led to extensive investigations into the endocrine-disrupting effects of chemicals in vertebrates. However, while invertebrates constitute over 95% of the known animal species, and maintain invaluable societal and ecological roles, little is known of the susceptibility of these organisms to endocrine disruption, especially to chemicals known to have androgenic effects in vertebrates. This study aims to determine possible endocrine disruptive effects of the androgen 17

TU004
Reproductive strategy and population dynamics of Hyalella azteca (Crustacea: Amphipoda) in culture conditions.
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Considering the importance of *Hyalella azteca* in ecotoxicological essays to assess environmental quality, this work aims to analyze the biological cycle and population dynamics of a population cultivated in LEAL (Laboratory of Ecotoxicology and Environmental Microbiology Prof. Dr. Abílio Lopes) Unicamp, Limeira – SP. Age-class structure, fecundity (average number of eggs produced), viability (average number of young released), generation time, reproductive potential, sex ratio, net reproductive rate, survival rate and intrinsic rate of population growth will be established experimentally. Embryos from 20 sexually mature pairs of *H.azteca* already in copula amplexus were randomly select from the existing culture tanks. After male fertilization and female release, the male was removed. 10 females that released their offspring in the nearest time interval were considered as the parental generation, and the brood called First Generation (G1) and their survival and reproduction rate monitored. Preliminary reproductive potential is 31,870, the generation time 2,99 and the per capita rate of population growth, 0,034. No sex ratio biased in favor of females or males was recorded in all sampling dates, denoting a not stressful condition. It has a Type II survivorship curve consistent with parental care. Taking in account the vital parameters assessed, endpoints will be suggested for toxicity testing with a population approach and referrals for monitoring the well-being of populations in cultivation.

TU005
Metal tolerance inheritance may unveils possible patterns of microevolution in amphibians populations
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Chemical contamination may lead to the occurrence of genetic erosion in natural populations, and, consequently, may impair their viability under future environmental perturbations. This is of particular concern if the loss of genetic variability is irreversible due, for example, to the contaminant-driven elimination of alleles when chemical tolerance is a fully or incomplete recessive (or incompletely dominant) trait – the recessive tolerance inheritance hypothesis (*sensu* Ribeiro and Lopes, 2013). Accordingly, this work aimed at investigating metal tolerance inheritance in natural populations of the frog species *Pelophylax perezi*.
Twenty-one egg masses, in Gosner stage 8-10, were collected from a population inhabiting a metal free pond and exposed to a metal rich acid mine effluent plus to a control (FETAX medium). Time to death was registered for each egg, with observations following a logarithmic time scale: 12h00min, 16h57min, 23h57min, 33h49min, 47h46min, 67h29min and 95h19min. For each egg mass, the median

lethal time (LT_{50}) and respective quartiles (LT_{25} and LT_{75}) were computed. Subsequently, critically sensitive egg masses (categorized as those with an LT_{75} similar or below the average of the set of LT_{50} for all egg masses) were identified. The within egg mass variability in time to death responses was evaluated through its relative spread: the difference between the lower and upper quartiles relatively to the median: $[(LT_{75}\text{-}LT_{25})/LT_{50}]$. If metal tolerance corresponded to a recessive trait then the most tolerant egg masses (both parents being recessive homozygotes) would present relative spreads lower than at least some of those with an intermediate LT50 (both parents being heterozygotes), which was not the case: no relationship whatsoever between relative spreads and LT50 values could be found. Also, only one egg mass was identified as critically sensitive and its relative spread was not particularly low. Therefore, the recessive tolerance inheritance hypothesis was not supported by the present study.

TU006

Variability of Cd-sensitivity and phylogenetic diversity of field populations throughout the Gammarus fossarum/pulex species complex.

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Gammarids from the *pulex* group (*e.g. G. pulex, G. fossarum*) are commonly used in ecotoxicological tests (both for laboratory and *in situ* assessment of contaminant toxicity). But this taxonomic group is known to be a highly diversified complex of cryptic species, and affiliation to a cryptic lineage is claimed to be a driving factor for toxicant sensitivity. On one hand, this could contribute to the reported between-population variability in response to contamination in gammarids, and on the other hand, it challenges the representativeness of toxicity tests performed with one experimental population. However studies addressing this issue are often based on few populations, while a quantitative knowledge of the natural range of between-population variability of sensitivity to toxicants is necessary to conclude about the potential confounding effect of the phylogenetic signal in this species group. Unfortunately, such a reliable quantification is often lacking. To answer these questions we proceed here in three steps. First, a broad sample of 18 field populations of *G. fossarum/pulex* complex has been selected. Particular attention was paid to the selection of populations inhabiting pristine stations spread out at a regional scale taking into account confounding factors. Second, we established lethal responses to cadmium for each population (time- and dose-response curves). Third, we investigated the genetic diversity among and within populations by evaluating nucleotide sequence variation in a neutral marker (COI). From this, the variability of acute Cd-sensitivity appeared to be weak. Genotyping and phylogenetic analysis revealed that our sample of populations covers a wide diversity in the *G. fossarum/pulex* species complex, notably in lights of reported sequences in literature. Indeed, a high haplotype diversity within and among study populations with several cryptic lineages that can occur sympatrically have been identified. Genetic divergence between cryptic lineages revealed in our study are comparable to interspecific level. Overall, our results sustain that in the particular case of acute Cd toxicity, the phylogenetic signal within the *Gammarus fossarum* species complex is very weak, and that cryptic interspecies heterogeneity does not constitute a systematic bias in toxicity assessment. In contrast local ecological adaptations should be investigated to understand potential between-population heterogeneity in sensitivity to contaminants in gammarids.

TU007

Adaptive Plasticity of Laguncularia racemosa in Response to Different Environmental Conditions. Integrating Chemical and Biological Data by Chemometrics

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Mangroves are dynamic environments under constant influence of anthropic contaminants. The correlation between environmental contamination levels and possible changes in the morphology of plants, evaluated by multivariate statistics helps to highlight matching between these variables. This study aimed to evaluate the uptake and translocation of metals and metalloids in roots and leaves as well as the changes induced in both anatomy and histochemistry of roots of *Laguncularia racemosa* inhabiting two estuaries of Espírito Santo (Brazil) with different pollution degrees. The analysis of 14 elements in interstitial water, sediments and plants followed by multivariate statistics, allowed the differentiation of studied sites, showing good match between levels of elements in the environment with the corresponding in plants. *L. racemosa* showed variations in their root anatomy in different collection areas, with highest values of cortex/vascular cylinder ratio, periderm thickness and air gap area in Vitória Bay, the most polluted sampling area. These three parameters were also important to differentiate the mangrove areas by linear discriminant analysis (LDA). The development stage of aerenchyma in roots reflected the oxygen availability in the water, being found a negative correlation between these variables. The combined use of chemical and biological analyses responded quite well to different pollution scenarios, matching morphological responses to physical and chemical parameters, measured at different partitions within the estuary. Thus, *L. racemosa* can be confirmed as a reliable sentinel plant for biomonitoring of estuaries impacted by anthropic pollution. **Keywords:** Chemometrics, estuary, contaminants, aerenchyma. *Acknowledgement* - The authors thank to Prefeitura de Vitória/ES, CNPq and CAPES.

TU008

Evidence for self-sustaining populations of wild roach in rivers where feminization of males is widespread

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There is substantial evidence for widespread femisation of male fish living in rivers contaminated with wastewater treatment works (WWTW) effluents. This has been attributed to exposure to natural and synthetic steroid estrogens, which are biologically active at very low (ng/L) exposure concentrations, including 17 alpha-ethinylestradiol (EE_2), a component of the contraceptive pill. Some of the best evidence for effluent-induced feminsation comes from roach (*Rutilus rutilus*) living in English rivers, yet the full implications at a population-level are not well understood. We examined whether the sizes of breeding populations of roach are substantially decreased in rivers with a high proportion of estrogenic effluent. Population genetic structure was investigated by examining variation at 14–19 DNA microsatellite loci in 1769 fish sampled between 1995 and 2011. To test whether WWTW effluents substantially reduce the size of breeding populations,effective population sizes (N_e), which relate to the number of breeding fish and skews in breeding success, were estimated using the microsatellite genotypes using approximate Bayesian computation. Population-genetic analysis revealed significant genetic structuring in roach populations in Southern England, including within the Thames Catchment. This suggests the existence of local subpopulations exchanging a limited number of effective migrants (breeding individuals). Furthermore, some populations have been confined to stretches of river with a high proportion of estrogenic effluent for multiple generations, and have survived, apparently without reliance on immigration of fish from less polluted sites. We found no evidence for a correlation between N_e and E2 equivalents (E2Eq), a measure of total estrogenicity of the river water due to contamination by sewage effluent (GLM, $F^{(1,20)} = 0.7468$, $p = 0.40$) across the 28 sample sites where no recent restocking had occurred and had sufficient sample sizes for robust N_e calculation. However a reduction in N_e of 65% is still possible for the most contaminated site because of the wide confidence intervals associated with the statistical model. These results demonstrate that roach populations living in some effluent-contaminated river stretches, where feminization is widespread, are self-sustaining. This raises the question of whether roach can, and have, adapted to the harmful effects of exposure to oestrogens.

TU009

A multigenerational perspective on perfluorinated compounds toxicity: life-history and genetic effects on Chironomus riparius (Diptera, Chironomidae)

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Perfluorinated alkylated compounds (PFASs) represent an emerging group of contaminants with peculiar physico-chemical and toxicological properties. PFASs are generally persistent in the environment and have been detected extensively in most parts of the aquatic and terrestrial ecosystems In this study, we investigated the long term sublethal effects of PFOS, PFOA and PFBS on the genetic and life traits parameters of *Chironomus riparius* (Diptera, Chironomidae) populations under a multigenerational assay. Starting from a common wild stock, 8 different subpopulations of *C. riparius* were breeded in separate vessels, each containing a 10 μg/l solution of PFOS, PFOA and PFBS or reconstituted water. A total of 300 midges were breeded for each cage for multiple generations . For each generation, the following life traits were estimated: survival, growth, development, reproduction. Heterozigosity, allele diversity, deviation from Hardy-Weinberg equilibrium, linkage disequilibrium, effective genetic dimension, population size

contraction and presence of selection was estimated basing on five microsatellites loci. The replicates which fulfilled the validity criteria for mortality and emergence according to OECD guideline 219 protocol were considered valid. No effects on survival were found, while sub-lethal effects were shown for PFOS and PFOA treatments, in terms of development rate, EmT50 and growth parameters. PFBS affected mainly the last generations. Overall, a general decrease of genetic variability was observed for all the replicates, indicating that breeding conditions and genetic isolation of populations favored genetic drift. Pattern of alteration of mutation rates was suggested in PFOS and PFBS exposed populations. In both the case, any indications of selection acting on genes linked to the analyzed microsatellites loci was excluded, although life-traits variations indicated potential adaptation of PFOS exposed populations. In the case of PFBS, mutational load may be at the base of the increased sensitivity indicated in life-traits analysis starting from the 6th generation. Both genetic and life history approaches indicated more relevant effects of PFOS, while minor effects may impair long-term persistence of natural populations exposed to PFBS. On the contrary, effects caused by PFOA on growth and development, but not on reproduction, did not reflect in genetic alterations, indicating the presence of non adaptative responses.

TU010

Metallothionein gene expression differs in earthworm populations with different exposure history

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Metals are persistent pollutants in soils that can harm soil organisms and decrease species diversity. Animals can cope with metal contamination with the help of metallothioneins, small metal-binding proteins that are involved in homeostasis and detoxification of metals. We studied the expression of metallothionein with qPCR in a small, epigeic earthworm, *Dendrobaena octaedra*. We compared expression patterns and metal body concentrations in earthworms collected from two sites differing in metal contamination: Harjavalta, contaminated by a Cu-Ni smelter, and Jyväskylä, an uncontaminated site. Earthworms from both sites were also exposed to different concentrations of Cu (control, 50, 100 or 200 mg/kg) or Zn (control, 75,150 or 300 mg/kg) for 7, 14 or 28 days to find out if there is a time related dose response in gene expression. Population comparison showed that earthworms from the two populations differed in metallothionein expression, being higher in earthworms from the contaminated site. In the exposure experiment, exposure time affected expression, but only in the earthworms from the uncontaminated site, suggesting that there is a delay in the metallothionein response of earthworms in this population. In contrast, earthworms from the contaminated site showed constant levels of metallothionein expression at all exposure concentrations and durations, which was higher than that in earthworms from the uncontaminated site. The constant metallothionein expression in earthworms from the contaminated site suggests that exposure history should be taken into account when metallothionein is used as a biomarker in the laboratory. Moreover, the results suggest that earthworms without previous exposure history would be better for such studies. Lack of a dose-response could that this isoform of metallothionein is not an accurate biomarker suited for monitoring Cu or Zn contamination. Adaptation of *D. octaedra* to metal exposure could explain the differences between the populations and explain the persistence of this species in contaminated forest soils.

TU011

Ecological and evolutionary impact of environmental acidification on amphibians

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Environmental acidification, such as acid rain or CO_2 - induced ocean acidification, can occur rapidly making it difficult for most species to adapt. As environmental stress can have strong ecological and evolutionary consequences on natural populations, it is of key interest how organisms cope with stressful environments. However, current studies on organismal effects of acidity are mainly short term experiments which test the sensitivity of the organism, and empirical data whether and how organisms adapt to acidity is highly desired. Here we present data for natural populations of two amphibian species (*Rana arvalis* and *Rana temporaria*), which show intra-specific adaptive divergence to acidity via maternally derived egg capsules. Particularly, we use cutting edge molecular approaches (proteomics, glycan analysis) to elucidate the molecular mechanisms behind adaptive maternal effects in these systems, and emphasize the role of egg capsules for adaptation at early life-stages.

Fish model species in environmental toxicology (P)

TU012

Pathology Working Group Review of Histopathologic Specimens from Three Laboratory Studies of Diclofenac in Trout

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Background: A Pathology Working Group (PWG) panel evaluated histopathologic sections from three prior studies of diclofenac in trout (Hoeger et al. 2005, Mehinto et al. 2010, Memmert et al. 2013). Data of a fourth pivotal trout study (Schwaiger et al. 2004) were unfortunately not available for review. Methodology: Following a complete examination of all histologic sections and original diagnoses by a single experienced fish pathologist (pathology peer review), a two-day PWG session was conducted to allow members of a four-person expert panel to review a subset of the histologic sections and determine the extent of treatment-related findings in each of the three trout studies. In accordance with standard procedures, the PWG review was conducted by the non-voting chairperson in a manner intended to minimize bias. The four voting panelists were unaware of the treatment group status of individual fish and the original diagnoses associated with the histologic sections. Results: Based on the results of this PWG review, findings related to diclofenac exposure included minimal to slightly increased thickening of the gill filament tips in fish exposed to the highest concentration tested (1000 μg/L), plus a previously undiagnosed finding, decreased hepatic glycogen, which also occurred at the 1000 μg/L dose level. The panel found little evidence to support other reported effects of diclofenac in trout, and thus the overall NOEC was determined to be 320 μg/L. On the other hand, the panel noted diagnostic inconsistencies within and among the previous studies, including a lack of diagnoses in the control group in one study, and technical shortcomings in another case. Conclusions: This exercise clearly demonstrated the importance and added value of using the pathology peer review / PWG approach to assess the reliability of histopathology results that may be used by regulatory agencies for risk assessment purposes. The analytical power of a PWG is far greater than that of a journal peer review in which the actual histologic slides are not available for examination. For the reliable derivation of Environmental Quality Standard (EQS) values, a PWG approach is highly recommended.

TU013

A test design to assess recovery in fish full lifecycle studies

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Fish full lifecycle (FFLC) tests are required when toxicity, bioaccumulation, and persistence triggers are met (in the EU), Estimated Environmental Concentration ≥ 0.1 x Fish early lifestage No Observed Effect Concentration (NOEC) (in the US), or when there is a suspicion of potential endocrine disrupting properties. Fish are exposed over at least one lifecycle (egg to egg) and increasingly over multiple generations under constant exposure conditions. However, for certain substances such as Plant Protection Products (PPPs), aquatic exposure in the real world will fluctuate following application regimes and the physico-chemical properties of the active substance. Therefore, the environmental realism of FFLC test exposure regimes is questionable. Here we describe a FFLC test design that incorporates recovery (transfer to clean water) groups after both short-term and life-long exposure. The test design is practical and does not significantly increase the number of animals used. Data from a fathead minnow (*Pimephales promelas*) study with a PPP are presented. These data demonstrate recovery in reproductive parameters. The value of such recovery data are discussed for environmental risk assessment, calibrating/validating toxicokinetic-toxicodynamic models and characterizing the severity of effects.

TU014

Protein Interactions in the Cascade of the Aryl-Hydrocarbon-Receptor during Embryogenesis of the Zebrafish (Danio rerio)

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-------The present study is a consequence of specific questions about molecular processes in the early development of zebrafish that have directly arisen from remarkable results of the joint research project DanTox. This project aimed to develop eukaryotic test systems which can be used to investigate the ecotoxicological effects of contaminated sediments on cell mechanism-specific and gene expression level using zebrafish. Gene expression analyses of the phase I metabolism demonstrated a significant decline of several CYP1 genes at 72 hours post fertilization (hpf) after an exposure of an equally lasting period to β-naphthoflavone (β-NF) which is well known as a strong inducer of the arylhyrdocarbon-receptor (AhR). At the same exposure time a proteome analysis revealed a significant reduction of the heatshock protein 90β (HSP90β). Other studies showed that HSP90β is highly relevant for the binding ability and specificity of the AhR thus being involved in organism's reaction to chemical exposure, too. In order to gain a deeper insight into these processes the present investigation is going to examine the influence of particular environmentally relevant pollutants in the signaling pathway of the AhR during embryogenesis of the zebrafish. The receptor is an important protein in the cascade of elimination and biotransformation of xenobiotika and hence in many cases responsible for their

effect. Therefore, recently fertilized embryos of the zebrafish are exposed to AhR-inducers (β-NF and PCB 126) till a maximum age of 120 hpf. Every four hours the amount of mRNA is measured by qPCR. The selected genes refer to proteins that take part in the AhR cascade (e.g. HSP90β, AIP, ARNT, AhR-Repressor). By increasing the detailed knowledge on the interactions between the reaction-partners in the cascade of the AhR international standard test protocols like the OECD guideline 236 on the "Fish Embryo Acute Toxicity (FET) Test" will receive a stronger basis and relevance in the field of chemical testing. In addition, by increasing the reliability of such test systems that are not considered as animal experiments will contribute to the establishment of rather molecular methods that can reduce animal experiments in testing and evaluating chemicals. The project is funded by the Deutsche Bundesstiftung Umwelt DBU.

TU015

Biomarkers of growth stimulants in Oreochromis mossambicus

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There has been an increasing concern worldwide regarding the possible adverse effects of pharmaceutical supplements on physiological functioning in humans and wildlife. Veterinary pharmaceuticals are provided as growth promoters in cattle feedlots. Therefore if released into the surrounding aquatic environment concerns about the possible harmful effects of these growth promoters on fish reproduction and general health developed in recent years. Livestock producers use androgenic and estrogenic growth stimulants to increase meat production and the efficiency of converting feed energy into meat. Residue amounts of the hormones were not restricted to the meat consumed by the user, but is also excreted in cattle manure from where it can contaminate surface and ground water. More information on the effect of these stimulants on the health and survival of aquatic organisms is needed. The physiological effects thereof on fish are useful bio-indicators of stress induced by the presence and increase in concentration of these growth promoters. *Oreochromis mossambicus was* exposed to Trenbelone acetate (14 and 15 μg/l), methyltestosterone (7 and 7.5 μg/l), diethylstilbestrol (0.28 and 0.29 μg/l), zeranol (2.8 and 3μg/l) and combinations of these estrogens and androgens in a flow-through exposure system. Fish were exposed under controlled laboratory conditions for 24-hours, 4-, 15- and 30-days respectively. In this study, Cellular Energy Allocation (CEA), Glutathione-S-transferase (GST) and Uridine-Diphosphate glucuronosyltransferase (UDPGT) assays have been used to assess the effects that the growth stimulants used have on the fish at cellular level. Muscle was used to determine the CEA, liver and kidney tissue were used for both GST and UDPGT assays. Standard techniques were employed. Minor decreases in CEA values and Zeranol prompted a significant increase ($p< 0.05$) in GST activity in the kidney after 4- and 15-days of exposure while no change was displayed in the liver values. The results are discussed in view of the importance of the findings to assist in the management of the effluent from cattle feedlots. The validity of biomarker responses in toxicity testing as a component of water quality monitoring programmes is considered. Key words: Biomarkers, fish, growth stimulants, feedlots

TU016

New transgenic zebrafish models to study the expression of key steroidgenic enzymes and their perturbation by endocrine disrupting chemicals

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Concern about the effects of Endocrine Disrupting Chemicals (EDC) to fish reproductive health has stimulated the development and implementation of fish screening and testing procedures for EDCs which has become an important aim notably within the perspective of the EU regulatory framework for chemicals REACH (registration, evaluation, authorization and restriction of chemicals). In this context, small fish species such as the zebrafish had appear as relevant models to identify endocrine active substances, quantify their effects and explore their modes of action. In this context, transgenic zebrafish can provide suitable and practical biological models to study EDC while reducing costs and the number of animals. For instance, we recently demonstrated the usefulness and relevance of a vivo mechanism-based test (the EASZY test that uses transgenic cyp19a1b-GFP zebrafish embryos) for rapid and cost-effective screening of estrogenic activity of chemicals. In the present work, our aim was to develop a panel of new transgenic models to study the expression and the perturbation of several target genes involved in the endocrine system and known to be affected by exposure to EDC. These new transgenic zebrafish lines express Green Fluorescent Proteins (GFP) under the control of the zebrafish promoters of steroidogenic genes, cyp11c1 and cyp19a1a. These genes are known to play a critical role in the biosynthesis of androgens and estrogens respectively and are affected by exposure to EDC. We found that the transgenic cyp11c1-GFP and cyp19a1a-GFP lines are homozygous resulting in 50% of transgenic embryos when crossing transgenic animals with wild-type fish. Furthermore, the transgene is stably expressed across generation (>F3). Extensive immunohistochemistry experiments showed that there is a perfect co-expression of GFP with endogenous zebrafish Cyp11c1 and Cyp19a1a proteins in the gonads. In both transgenic lines, GFP was localized in the cytoplasm of oogonia, young oocytes and peri-follicular cells and in testes, GFP was localized in the cytoplasm of Leydig cells and germ cells. Monitoring the expression of these transgenes on the whole animal or on sections in control and exposed-fish will help to identify the interest of these models to study critical physiological process (e.g., sexual differentiation). Since the fish brain is characterized by a strong ability to synthesize neuro-steroids, their expression in the central nervous system will also be considered.

TU017

Combined effects of estrogenic ligands on zebrafish specific in vitro and in vivo bioassays

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To date, studies on endocrine disruptors mixtures have mainly focused on the potential additive effects of estrogenic compounds. Until now, most of *in vitro* and *in vivo* assays that have been used to address mixture effect in fish are based on ERα and liver vitellogenin expression, while mixture effect of estrogens on other ER subtypes and other ER-regulated genes is still poorly explored. In this context, the aim of our project is to determine the effects of binary mixtures of estrogenic compounds on the three zebrafish ER subtypes and on the brain specific ER-regulated *cyp19a1b* gene. For that purpose, newly established zebrafish-derived tools are used: (1) *in vitro* stable reporter gene assay (ERE-luciferase/ zfER-α, -β1 or -β2) in a zebrafish liver cell line (ZFL) (2) *in vitro* transient reporter gene assay (ZfCyp19a1b-luciferase/ zfER-α, -β1 or -β2) in a human glial cell line (U251-MG) and (3) *in vivo cyp19a1b*-GFP transgenic zebrafish. Concentration-response relationships for all single chemicals were measured, modeled, and used to design the binary mixture experiments following a ray design. The results from mixture experiments were analyzed to predict joint effects according to concentration addition (CA) and independent action (IA) models. Two different statistical approaches (Jonker's dose-response surface models and Hewlett and Streibig's isobole-based models implemented by Sorensen) were used to assess deviations from the CA model and to characterize interactions between the components of the mixtures (synergism/antagonism). Our study showed that binary mixtures of model estrogenic compounds generally lead to additive effects, even if some deviations could be observed. As regards binary mixtures of estrogenic compounds with different estrogenic potency, our study highlights an antagonism between E2 and genistein in mixture both *in vitro* (U251-MG) and *in vivo* in a glial cell context. To our knowledge this is the first report of an antagonism between these two compounds in brain context. Conversely, in another cell context, i.e. the liver (ZFL cells), preliminary results on the effect of mixtures of E2 and genistein showed a slight synergistic effect between the two compounds. The future development of our work will concern the effects of mixtures of estrogenic compounds with indirect estrogenic ones(needing metabolization prior to elicit estrogenic activity). **Endocrine disruptors, mixtures, zebrafish\n \n**

TU018

Steroid and thyroid hormone levels in trout plasma determined by LC/MS/MS

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Steroid and thyroid hormone plasma levels of fish can be affected by endocrine disruptor contaminants present in water or feed. A method by LC/MS/MS for the simultaneous determination of fifteen fish hormones in plasma of trout (*Salmo trutta*) was developed. The method was used to determine during a reproductive cycle the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), the glucorticoids hydrocortisone and aldosterone, the progestagen progesterone, the androgens 11-ketotestosterone, 17α-methyltestosterone, 4-androstene-11-β-ol-3,17-dione, adrenosterone, testosterone and 4-androstene-3,17-dione and, the estrogens estrone (E1), estriol (E3), 17α-estradiol (αE2) and 17β-estradiol (E2). Blood samples of eight anesthesized and identified fish (500-1000 g) have been collected by venipuncture from the caudal vein, monthly from September 2012 to March 2013. The fish were maintained in a tank at the Technical Superior School of Forest Engineers (Polytechnic University of Madrid). Hormones were extracted from plasma samples after a clean-up in OASIS HLB Waters (Mildford, MA, USA) cartridges. The methanolic eluent was dried. The extract was reconstituted in acetonitrile/water (10/90) and passed through 0.2 μm filters before its injection in the chromatographic system (Infinity 1260

HPLC-System with a 6420 Triple Quad LC-MS/MS (ESI) detector, Agilent Technologies Inc., USA). Chromatographic separation was achieved with a C18 column using a water-acetonitrile with 0.15 % formic acid mobile phase gradient and 0.2 ml/min flux. This method allowed the determination of 14 hormones in 42 min. Although hydrocortisone could be detected, its quantification was not possible due to matrix effects. The method allowed detecting these hormones at limits of 0.5 – 5 ng hormone / ml plasma with limits of quantification (LOQ) in the range of 2 – 5 ng/ml. The recoveries at the LOQ and 70 ng/ml levels ranged from 79 to 111%. The maximum concentrations of T3, T4 and the androgens were observed in November. Hydrocortisone could be detected in all the samples whereas progesterone, E1, αE2 and aldosterone were not found in any of the samples. E3 and E2 were only detected at trace levels in some of the samples, indicating that the chosen animals were still immature.

TU019

Morphological and behavioural effects of teratogenic compounds in the zebrafish embryo show different mechanisms of teratogenicity

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Within the REACH legislation there is a priority for reproductive toxicants. Many studies have examined the suitability of zebrafish embryo assays to predict the teratogenic potential of chemicals in mammals. Zebrafish are not considered test animals until 120 hours post fertilization (hpf) according to European legislation. Therefore, zebrafish can be used in *in vivo* alternative testing strategies. Moreover, it offers the physiological relevance of a vertebrate whole-organism test system relative to *in vitro* alternatives. However, it has become clear that the mechanistic basis of teratogenicity is still unclear and should be investigated further in order to allow for accurate identification of teratogens. Morphological deviations are often used for this purpose. By including behavioural parameters, we believe to significantly advance this model system and its potential to study developmental defects. In this study, zebrafish embryos were exposed to caffeine, cadmium, hydroxyurea, retinoic acid and warfarin sodium until 96 hpf. At 72 and 96 hpf, the behaviour and length was assessed along with a scoring of 30 morphological endpoints. The results showed that measurements at 96 hpf provided more mechanistic information compared to 72 hpf. There were important differences in the responses to the tested teratogens. Warfarin sodium induced the highest number of effects including a lack of blood circulation in the tail, blood accumulation in the yolk, malformations of the tail and a decrease in length and hatching. Furthermore, a decrease in the number of movements, duration of the movements, swimming distance, speed and number of clockwise and counterclockwise turns could be observed. Caffeine induced mostly malformations of the tail, non-inflated swim bladder and a decrease in similar behavioural parameters as warfarin sodium, except for swimming speed which was not affected. Also cadmium induced non-inflated swim bladders, accompanied by a number of cardiovascular defects. However, less behavioural effects could be observed. The most apparent effect of retinoic acid and hydroxyurea was blood accumulation in the pericard, but no significant effects on swimming speed and activity could be detected. These results demonstrate that chemicals can cause teratogenicity by multiple mechanisms, resulting in different behavioural and morphological response patterns. This should be taken into account when using the zebrafish embryo as a teratogenicity screening tool.

TU020

A test battery for the screening of neurotoxicity of anthropogenic trace substances in aquatic ecosystems

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D. Stengel, Th. Braunbeck, Aquatic Ecology & Toxicology, Univ. of Heidelberg, INF 230, D-69120 Heidelberg Water purity is central to the health of both environment and man all over the world. Therefore, an integrated research program, "Risk Management of Emerging Compounds and Pathogens in the Water Cycle" (RiSKWa), was initiated by the German Federal Ministry of Education and Research (BMBF) to promote and secure water quality and safety. The associated project ToxBox is designed to develop guidance for the risk assessment of anthropogenic of trace substances the data base for which is insufficient. The ToxBox project "*in vivo* neurotoxicity" is based on a battery with 4 different neurotoxicological endpoints in zebrafish embryos: (1) olfaction *via* whole-mount staining with cell specific antibodies, (2) vision *via* cryo-techniques and fluorescent staining of retina, (3) tactile sense *via in vivo* neuromast staining, and (4) synaptic transmission *via* classic enzyme biochemistry of acetylcholine esterase. Each endpoint could be shown to react to known (and suspected) neurotoxicants with high specificity. Acetylcholine esterase, e.g., exclusively reacted to known man-made AChE-inhibitors. For Dichlorvos, a significant decrease by 47 % could be shown from 0.75 mg/L. The resolution of the neuromast-assay could be increased by additional *in vivo* dapi-staining and enhancement of established scoring methods. Thus, toxicity to neuromasts by substances like neomycin and copper sulfate was made possible. In addition, the AChE-inhibitor dichlorvos could be shown to reduce neuromast fluorescence by 18 % at 0.75 mg/L. Whole-mount staining of the olfactory epithelium with cell specific antibodies (anti-$G_{alphaSolf}$) proved to be suitable for structural modifications; yet, neither cell- nor antigen-specific effects could be detected at concentrations tested so far. Overall, the battery based on the combination of structural modifications of neurological systems and functional parameters appears to be quite specific in its reactivity. So far, it has been demonstrated to be capable of detecting both neurotoxic heavy metals and specific AChE-inhibitors. This project is foundet by the German Federal Ministry of Education and Research (BMBF) FKZ:02WRS1282G

TU021

Effects of barium carbonate and barium chloride on Daphnia magna, Moina macrocopa and reproduction of zebrafish

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Barium found in the aquatic environment has been of growing concern, but few investigations have been performed to examine the potential consequences on ecosystem heath. In this study, chronic effects of barium carbonate ($BaCO_3$) and barium chloride ($BaCl_2$) were evaluated using *Daphnia magna* and *Moina macrocopa*. Adult zebrafish pairs were also exposed to sub-lethal concentrations of $BaCl_2$ for 21 d, and the effects on reproduction, sex steroid hormones, and transcription of the genes belonging to the hypothalamic-pituitary-gonad (HPG) axis were investigated. The adverse effects on performances of F1 generation were further examined with or without subsequent exposure to $BaCl_2$. *D. magna* and *M. macrocopa* demonstrated significant changes in reproduction (number of young per female) after exposure to $BaCO_3$ and $BaCl_2$. Egg production was significantly decreased at 0.1 mg/L $BaCl_2$. Parental exposure to $BaCl_2$ resulted in lesser rates of hatching even when they were hatched in clean water. Continuous $BaCl_2$ exposure in the F1 embryos resulted in worse hatchability and malformation rates compared to those without $BaCl_2$ exposure. Our observation showed that exposure to $BaCl_2$ and $BaCO_3$ could affect the reproduction and impair the development of offspring.

TU022

Effects of subchronic exposure to atrazine on selected indices in common carp (Cyprinus carpio L.)

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In the subchronic tests, fish were exposed to a range of four sublethal concentrations of test pesticides (0.3 – environmental concentration in the Czech rivers; 300; 1000 and 3000 µg/l). The experiment was conducted in the continuous flow system for 12 weeks. Concentrations of atrazine in water were quantified using LC/MS during the toxicity test. During the experiment (weeks 1, 3, 6, and 12), individual blood samples were taken by cardiac puncture and used for determination of basic biochemical and haematological parameters. The fish were killed, the body weight recorded, and liver, gills, kidney, skin and muscle were dissected for histopathological examination. During the test, the condition of fish was checked and there were detected some behavioral changes (decrease in food intake and body pigment) and parasite presence (*Ichthyophthirius multifiliis*) at the highest concentration of atrazine exposure (3000 µg/l). Because of adverse health status of the experimental group exposed to the highest concentration of atrazine, all fish from this experimental group were sampled during the sampling in 3th week and this group was canceled. This result confirms the immunosuppressive effects of atrazine and negative effects of high concentration of atrazine on health status of common carp. Histopathological examination revealed pathological lesions in pesticide-exposed fish in the experimental group with atrazine concentration of 1000 µg/l (only after 12 weeks exposure). Morphological changes were observed in the gills, represented by moderate multifocal teleangiectasis as a result of the rupture of the retaining pillar cells and dilation of the capillaries and pooling of the blood with formation of thrombi, hypercellularity and lamellar hyperaemia. Significant changes were also found in biochemical and haematological indices. This research was supported by GACR P502/12/P163.

TU023

Effects of Zinc exposure on the survival of Arabian Gulf Killifish embryos under variable saline conditions

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Fish embryos are excellent models for studies aimed at the understanding of toxic mechanisms and the indication of possible acute and chronic effects. For the past 3 years, Arabian killifish fish embryo test (mFET) has been developed in our

laboratory as a routine ecotoxicological test for risk assessment of potential contaminants in Arabian Gulf around Qatar. To further explore the applicability of the killifish embryos as a model for the Arabian Gulf conditions, we tested the toxicity of zinc (Zn) using artificial seawater with salinity level 40 ppt. which is the average salinity of Arabian Gulf seawater. Other experiments were performed at salinity level 20 ppt. as comparison. Embryos were exposed to Zn concentrations (0, 2.5, 5, 15, 25 and 35 mg/L). The tests studied embryogenesis success from fertilized egg to normal larvae. The results showed that Zn toxicity decreased at increased salinity. At salinity 40 ppt. Zn had no effect on mortality and hatching in any of the tested concentrations. In comparison, embryos exposed at salinity 20 ppt. showed higher mortality and malformations compared to the control at concentrations > 5 mg/L Zn. Examination of the existing literature on acute Zn toxicity to fish and larvae showed similar results to the findings in this study using killifish embryos. This clearly indicates the suitability of the killifish embryos toxicity tests in acute toxicity tests of trace heavy metals. The findings further support the ability of Killifish to be an indicator organism for environmental risk assessments of Qatari waters. Benefits include; sensitivity to wide range of substances and conditions, animal alternative, ease of fish breeding, clarity of the embryos, reduced sample size, reduced waste generation and shorter study duration.

TU024

Effects of organic compound Phenanthrene and Naphthalene on zebrafish (Danio rerio) early-life stages

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The Fourth Scientific Report of the IPCC AR4 presents evidence of climate changes that could significantly affect the planet, specifically in developing countries from tropical regions. The Amazon Basin holds the greatest diversity of fish in the world, and their distribution across the myriad of habitats in the Amazon region is defined by the complex organism-environment interactions. In the Amazon region there are major sources of pollution due to anthropogenic activities, namely associated with high concentrations of metals reaching water bodies and the presence of oil refineries in the river basin. Phenanthrene and naphthalene are PAHs that can contaminate the environment. They are known to cause toxic effects in aquatic organisms although risks to tropical fish are not well known. The objective of this study is to evaluate the acute toxicity of phenanthrene and naphthalene of zebrafish early life stages and quantify biochemical biomarkers (GST, LDH and CHE) as indicators of exposure to these PAHs. The exposures were conducted following the OECD's protocol for the Fish Embryo Toxicity (FET) Test, 2006. To determine the toxicity of Phenanthrene for early-life stages of zebrafish the following treatments were used: 0 (control), 0 + (solvent control) (3.750 ml L^{-1} of DMSO), 1.5, 2.2, 3.2, 5.0 and 7.5 mg L^{-1} of phenanthrene and 0 (control), 0 + (solvent control) (0,453 ml L^{-1} of acetone), 14.5, 25.0, 45.0, 80.0 and 145.0 mg L^{-1} of naphthalene. Biomarkers' activity (GST, AChE and LDH) was measured in early-life stages after exposure to concentrations of phenanthrene and naphthalene corresponding to the LC1, LC2, LC5 and LC10. The lethal concentration for 50% of the population after 96hours of exposure ($LC_{50\text{-}96h}$) was calculated with the help of ToxCalc spreadsheet under Microsoft Excel software. One-Way ANOVA test was used for data analysis of enzyme activity with SigmaPlot 11.0. $LC_{50\text{-}96h}$ for the zebrafish early-life stages. The parameters assessed in this study will contribute to the understanding of the mechanisms of action and toxicity of phenanthrene and naphthalene in *Danio rerio*. **Topic:** – Aquatic and Terrestrial Ecotoxicology **Keywords**: Fish embryo toxicity test, acute toxicity, biomarkers;

TU025

EVALUATION OF TOXIC EFFECTS OF DETERGENT IN EMBRYOS AND ADULT ZEBRAFISH Danio rerio

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In ecotoxicological studies, the zebrafish has been used as a test organism for assessing the effects of chemical compounds mainly. In toxicity tests, has been used to embryos of this species because its response comparable to the sensitivity of adult organisms. In Mexico, the use of these organisms is not stipulated in the legislation, despite being an alternative to replace fish bioassays. The aim of this study was to determine the toxic effect of two surfactants: LAS and Triton X and 5 products (Extram, Ace, Ariel, Foca and Roma) in embryos and adult zebrafish to compare their sensitivity. Static bioassays were conducted with a duration of 48 hours, embryos and adult fishes were exposed to 5 concentrations of toxic (12 replicas), plus a control without toxic. LC_{50} was determined and a comparison of the LC_{50} obtained to test are made to detect if the responses of embryos and adult fish are different. The toxicity of surfactants was (high to low toxicity): TX > LAS. And products: Extram > Ariel > Roma > Ace > Foca. Comparison of LC_{50} indicated no significant differences in the effects of detergents between embryos and adults in tests with Ace, Foca and Roma products. But if observed differences in the response of adults and embryos exposed to surfactant and the detergent Ariel. The embryos were more sensitive to these products. The results indicate that tests with zebrafish embryos are a useful tool in monitoring studies. Because wastewater discharges in the Valley of Mexico contain high concentrations of detergents (2-200 ppm) and their final destination is the aquatic systems, for this reason it is important to continue research to detect responses that indicate the possible adverse effect on fish by the action of different the discharges and tensors to prevent irreversible deterioration of the populations in the medium and long term.

TU026

Adverse effects of climate change related environmental parameters on zebrafish embryo development and survival

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Global warming is of high concern also in relation to chemical exposure. During the last century mean global temperatures have been increasing and consequently changes in several physico-chemical parameters (e.g. pH, dissolved oxygen) were observed in surface waters. Alterations in abiotic parameters of freshwater environments are likely to affect distribution, morphology, physiology and richness of a wide range of species leading to important changes in ecosystem biodiversity and function. Therefore, the main goals of this work are to evaluate stress tolerance of zebrafish (*Danio rerio*) embryos to variations in pH, dissolved oxygen and UV intensity as a basis to study interactions with chemical effects. Embryos were exposed to different ranges of the selected parameters and lethal and sub-lethal endpoints were recorded. Sublethal endpoints included hatching success, edemas, reduction of body length and alterations in heartbeat rates. Embryos were exposed to a pH range from 3 to 12 using different buffers (MES, TRIS, CAPS, CHES) in order to stabilize pH values. Effects due to low dissolved O_2 were assessed by exposing embryos to a 0 – 5 mg/l range. This was achieved by using a controlled atmosphere chamber with O_2 depletion by N_2 injection and posterior isolation in hermetic flasks. Finally, UV effects were assessed by exposing embryos continuously to UV doses ranging from 64 to 467 mW/m^2. UV dosage was accomplished by using acetate filters directly applied to the lamp. All the assays were conducted for 96 hour and analysis of effects in 24h intervals. Our data suggest that oxygen concentrations below 4 mg/L caused a lower hatching success and a delay in embryo development. Regarding UV exposure, malformation and high mortality rates were observed even for the lowest tested dose. pH values below 4.5 had significant effects on embryo survival. Our data provide a very useful basis for ecological risk assessment. However, for more environmentally relevant scenarios the interaction of a combination of environmental stressors and environmental pollution (e.g. pesticides) have to be considered – in order to estimate whether synergism may produce effects that can lead to unpredictable risks.

TU027

Combined effects of Ultraviolet radiation and xenobiotics on zebrafish embryos - changes in bacterial communities

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In their natural environment, organisms establish intimal associations with surrounding microbial communities, constituting their own microflora. Natural microflora plays an important role on host health, contributing to their immune system, being the "first line of defense" against pathogens, furnishing some nutrients that host could not access alone (e.g. vitamins, minerals and enzymes), conditioning behavioral patterns and weight regulation. In aquatic ecosystems some environmental parameters (e.g. Ultaviolet radiation, temperature, oxygen levels and pH) and/or pollutants can constitute stress factors weakening this "first line of defense" of the organism. There are now strong evidences suggesting that chemicals and environmental stressors can interact synergistically augmenting the expected toxicity for organisms; however the effects of these possible interactions have not been studied in organisms' natural microflora. With the global climate changes, it is expected that environmental parameters such as UV radiation may increasingly act as co-stressors. Thus, this work aims at studying the effects of combined exposure to UV radiation and three xenobiotics (triclosan (TCS), potassium dichromate (PD) and prochloraz (PCZ)) on bacterial communities associated to zebrafish (*Danio rerio*) embryos. Zebrafish embryos were exposed to three different intensities of UV radiation combined with three different concentrations of each chemical for 8 hours. After this period embryos were only exposed to the chemical, and at 48 hours post fertilization the molecular analysis (DNA extraction, polymerase chain reaction (PCR) and denaturing gradient gel

electrophoresis (DGGE)) was performed. DGGE provides information about microbial community diversity and abundance through the analysis of the number of bands and their intensity. Results showed an effect on microbial community diversity in all treatments. New bacterial community (new bands in the DGGE) seems to appear in embryos exposed to UV radiation, or TCS while PD showed to reduce the microbial diversity (almost completely in the highest concentration). PCZ did not induce a significant effect on microflora diversity. Combination of UV radiation and each of the chemicals produced different patterns of bands suggesting that interactions did occur.

TU028

Low doses of the herbicide ametryn induces oxidative stress in zebrafish embryos

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Ametryn (AMT) is the most widely used herbicide in sugarcane culture. It is known as a diffuse pollutant, being found in surface water and sediment of water bodies adjacent to the crops fields. In the present study, the effects of AMT on oxidative stress pathways were evaluated using zebrafish embryos. Embryos were exposed to 0, 4, 23, 190, 833, 5000, 10000 μg L^{-1} of AMT in 6-well microplates during 96 h. Different biomarkers of oxidative stress were analysed, namely catalase (CAT), lactate dehydrogenase (LDH), lipid peroxidation (LPO), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST). The AMT showed to be slight acutely toxic for zebrafish embryos with a 96 h-LC_{50} of 48.46 mg L^{-1}. Despite the low lethal toxicity embryos that survived to AMT exposure showed several developmental abnormalities including oedemas even at the lowest concentration of 10 mg L^{-1}. Effects on biomarkers activity of zebrafish embryos were observed in concentrations as low as 4 μg L^{-1} (LDH induction). Regarding oxidative stress biomarkers GR, GPx, GST and LPO were induced by AMT exposure whereas CAT was inhibited. Sub-lethal effects proved to be very sensitive and rather than lethality, are particularly relevant as they can be elicited by low doses and long-term exposure to AMT, which are more prone to happen in a real scenario.

TU030

Interactions between dietary methyl mercury and selenium affects selenoprotein gene expression.

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Elevated dietary levels of selenium (Se) can reduce methyl mercury (MeHg) toxicity, but the mechanisms behind this interaction are unclear. We explored selenoprotein gene regulation in relation to maternally transferred dietary Se (as selenomethionine) and MeHg in zebrafish (*Danio rerio*) embryos. Female adult zebrafish were exposed to dietary MeHg (12 mg Hg/kg) and/or selenium (10 mg Se/kg). Fertilized embryos from these females were analysed by real-time PCR for selenoprotein mRNA levels at 2 days post fertilization (dpf), and embryo locomotor activity was assessed at 3-5 dpf. The response of selenoprotein mRNA expression to MeHg and Se levels could be classed into one of three groups. The first group of genes did not respond to either MeHg or Se, including some involved in thyroid hormone metabolism (*dio2*), Se transport/storage (*sepp1a, 1b*), redox signaling (*txnrd3*), Sec synthesis (*sps2a*), or are uncharacterized (*selt1a*). The second group of genes were downregulated by MeHg but not affected by Se. This group included antioxidants (*gpx1b*), and statistical trends indicate it includes several other genes (*selh*; p=0.056, *dio1*; p=0.080, *txnrd1*, p=0.086). In the last group, downregulation by MeHg was prevented by additional Se, and included *gpx1a* and *4a*. Locomotor activity of the embryos was reduced (hypoactivity) by MeHg and partially reversed by additional Se. This indicates that MeHg only downregulates a subset of the selenoprotein genes. Se only prevented MeHg downregulation for two selenoprotein genes, and Se was only able to partially reverse MeHg induced hypoactivity. Interestingly the affect of MeHg on genes was not specific to any one functional group, while the preventative effects of Se were specific to genes encoding antioxidant proteins. This suggests that Se may prevent MeHg via maintenance of cellular redox balance, but may be less able to prevent MeHg induced gene regulation in other biologically important pathways such as thyroid hormone metabolism.

TU031

The effect of calcium on accumulation and toxicity of dissolved copper in the early stages of zebrafish embryo development: biochemical effects and gene expression

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Copper is an essential micronutrient, but elevated aqueous concentrations can be toxic in fish early life stages. The objective of this study was to assess the role of dissolved calcium during exposure to copper on the accumulation of total Ca^{2+}, Cu, K^+ and Na^+ in zebrafish embryos. In addition, the effects of exposure on total glutathione, Na^+K^+-ATPase activity, and expression of nkx2.5 (a gene important in initiating cardiac development) and Mt2 (metallothionein) gene transcripts were assessed. Embryos [age 1hour post fertilization (hpf)] were exposed to copper (0, 100, 250, and 500 μgl^{-1}), with or without added calcium (40 mgl^{-1}). Live and dead embryos were collected at 16 hpf. A significant (ANOVA, $P < 0.05$) increase in Cu accumulation was observed in both live (control, 0.0155 ± 0.001; Cu only, 0.108 ± 0.018; control, 0.0129 ± 0.001, Cu+Ca, 0.140 ± 0.023 $\mu molg^{-1}dw$), and dead embryos (control, 0.02 ± 0.001; Cu only, 0.48 ± 0.036; control, 0.03 ± 0.012, Cu+Ca 0.52 ± 0.015 $\mu molg^{-1}$ dw) exposed to copper with and without added calcium.The concentration of Ca^{2+} increased only in dead embryos (ANOVA, P< 0.05). Dead embryos had lower amounts of Na^+ and K^+ in all treated groups in comparison with live embryos. A 5 fold decrease in Na^+K^+-ATPase activity was seen in embryos exposed to copper compared to the control. There was no effect of calcium on total glutathione (ANOVA, P>0.05), but the expression of nkx 2.5 which is responsible for cardiac formation and development increased significantly; approximately 10 fold in the presence of Cu+Ca in comparison to the unexposed control or Cu exposure alone. The expression of Mt2 increased (ANOVA, P< 0.05) significantly 6 fold compared to the control during Cu exposure, but not with added Ca^{2+}.The results of exposure of embryos to copper for 16 h are consistent with disturbance of osmoregulation, and the addition of calcium to protect against copper toxicity. It is possible that Ca^{2+} modulates the expression of the nkx 2.5 gene, but only during Cu exposure, although the mechanism requires further investigation.

TU032

Zebrafish embryo as a sensitive model for the assessment of teratogenicity of cyanobacterial exudates containing retinoids

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Potent teratogenic compounds, namely retinoic acids and their derivates have been recently\n\nidentified by chemical analytics in cyanobacteria and algae. Retinoids are chemically related to \n\nvitamin A. Some of them are essential nutrients and deficiency or excess cause teratogenicity. This \n\nstudy used a testing strategy for prioritization of potentially teratogenic environmentally relevant \n\nmixtures followed by detailed assessment of selected samples. *In vitro* assay for identification of \n\nretinoid activity was combined with zebrafish embryo tests for determination of teratogenicity. \n\nOur study focuses on highly environmentally relevant exudates (extracellular compounds produced \n\nby phytoplankton cells). Exudates of ten cyanobacterial and algal species were screened *in vitro* \n\nfor their retinoid activity relative to all-trans retinoic acid (ATRA). Three species were selected \n\nbased on in vitro results for testing the teratogenicity of exudates on zebrafish embryos: the two \n\nmost potent species, cyanobacteria *Microcystis aerigunosa* and *Cylidrospermopsis raciborskii* and \n\none algae without retinoid activity. Embryos were also exposed to ATRA in the range equivalent \n\nto the concentrations as determined in the exudates. Teratogenicity was observed after exposure \n\nto exudates of cyanobacteria *Cylidrospermopsis raciborskii* starting at environmentally relevant \n\nconcentrations, and for *Microcystis aerigunosa* in threefold concentrated exudate. This corresponds \n\nto retinoid acid equivalent levels expected from the *in vitro* assays. Phenotypes caused by exudates \n\nwere similar to those caused by ATRA. For instance, an increased length of embryos was seen in \n\nlower concentration of exudates as well as in the corresponding concentrations of ATRA. The study \n\ndocuments that some cyanobacteria are able to produce and release retinoid compounds into the \n\nenvironment at concentrations equivalent to those causing teratogenicity in zebrafish. Since retinoid \n\npathways are highly conserved among vertebrates, these findings provide concern for impact on \n\nother aquatic vertebrates. The work was supported by the Czech Science Foundation grant No. GACR \n\nP503/12/0553.

TU033

Organic anion transporting polypeptide (Oatp) mediated uptake of cyanobacterial toxins into fish

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Many species of cyanobacteria can produce toxic secondary metabolites, which may result in acute poisoning of fish and contamination of drinking waters. Raising concerns arise from anthropogenic eutrophication and global warming of water bodies that promote the occurrence of cyanobacterial blooms and associated toxins, microcystins are the most abundant and most toxic cyanobacterial toxins. Their toxicity is mainly based on irreversible binding to phosphatase families PP1 and PP2A. To elicit their toxic effect, uptake into cells is necessary, however, a passive mechanism of membrane penetration was excluded. In mammals and a lower vertebrate, uptake by membrane transporters was shown, but uptake mechanisms in

teleost fish is unknown. The aim of our study was to characterize the uptake mechanisms and toxicity of different microcystin congeners and additional cyanobacterial toxins in fish by focusing on the specific zebrafish uptake transporter Oatp1d1. We developed a transgenic expression system using HEK cells stably expressing zebrafish Oatp1d1 for studying uptake of cyanobacterial toxins. Thereby we established an inhibition assay using a fluorescent substrate of the transporter. In addition, we used fluorescent labeled microcystin. We further compared the toxicity of microcystins and other cyanotoxins to Oatp1d1 transfected HEK cells and fish cells in relation to the amount of expressed Oatp1d1. Our study shows that Oatp1d is involved in the uptake of microcystins and additional cyantoxins in zebrafish.

TU034

Redtail splitfin (Xenotoca eiseni) as a potential new model for studies on maternal transfer of environmental contaminants

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Early life stages tend to be amongst the most vulnerable for adverse effects of chemicals. To date, models for studies on maternal transfer and effects of chemicals on developing early life stages have been focused on mammals. However, some fish species give birth to live young and offer favourable models for studies on maternal transfer. Here we investigated the reproductive biology of the redtail splitfin (*Xenotoca eiseni*), a viviparous freshwater fish of the goodeid family, with a view to develop this species as a model for studying maternal transport and embryo susceptibility to toxicants. We conducted studies to characterise the ontogeny of gonadal development, gestation and the process of embryogenesis in *Xenotoca eiseni*. Gonads were examined by light microscopy to stage sexual development from birth until sexual maturity. In a further study the progression of embryogenesis was examined in females throughout pregnancy. To do so, pregnant females were sacrificed and the ovaries dissected out, processed and examined by histology. At birth, embryo total body length was 13.5?0.9 mm (Mean?SD) and weight 30.6?7.1 mg. Gonads were fully differentiated and were seen as a two lobed structured testis joined at the anterior end, or a single lobed ovary, separated into two compartments by a highly folded septum. Females reached full maturity around 12 weeks after birth. The ovary is the site for egg production, internal fertilisation and gestation. Eggs were fertilised within the follicle, where the embryos were retained only for a brief period before they were discharged into the ovarian lumen. Two weeks after fertilisation embryos hatched within the ovary. By this time, trophotaeniae started to grow which implies that embryos were starting to depend on maternal provisioning. The ovarian lumen epithelium is known to be involved in the exchange of nutrients from the maternal vascular system into the ovarian fluid. Gestation normally took 6 weeks and the brood size was found to be highly variable, with the number of offspring produced in a single brood ranging between 1 and 44. During gestation of the developing larvae, oocytes continued to mature within the ovarian walls and female fish were ready to mate shortly after parturition. These studies provide the underpinning for the application of *Xenotoca eiseni* to maternal transfer studies of environmental contaminants and their effects in fish early life stages.

TU035

Medaka (Oryzias latipes) - A good fish model for testing of endocrine active substances

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Based on data generated in a Fish-Sexual-Development-Test (FSDT) using a peak-exposure design and in a 2 ½ multi-gen. Fish-Full-Life-Cycle test (FFLC) under chronic exposure the suitability of the Medaka for detecting endocrine active substances is presented and discussed with respect to other methods. Test substances were 4-*tert*-Pentylphenole (4tPP) and Trenbolone (TR). In the FSDT a peak-exposure at 3 different larval developmental stages was investigated. As it is discussed that for endocrine disruptors possibly already short term exposure is sufficient to cause adverse effects, this test was designed to gain more information on possible sensitive developmental life stages as well as for investigating whether the hypothesis is true in general. In addition a multi-gen. FFLC was performed to demonstrate that similar or even lower test concentrations were resulting in clear adverse effects. The comparison between the effects after chronic exposure was compared with the findings of the FSDT. For the FSDT and FFLC the standard endpoints (hatching-success, mortality and development) and the concentration of Vitellogenin (Vtg) were determined. Concerning the sex ratio related endpoints the phenotypic, histological and genetic sex were identified. Within the FFLC the reproduction of F_0 and F_1 were investigated. After peak-exposure tested in the FSDT no adverse effects were observed. In the FFLC a significant increase in mortality of F_0 was observed at ≥ 100 µg 4tPP/L. Females at 50 ng TR/L were significantly larger than in the control. An increase in the weight of females exposed to 400 µg 4tPP/L and males exposed to ≥ 200 µg 4tPP/L was observed. The Vtg-concentrations of males were elevated in treatment-groups ≥ 200 µg 4tPP/L. Feminization was observed in the phenotypic sex-ratio at 400 μg 4tPP/L and masculinization at 50 ng TR/L. A decrease in the number of eggs/female*d^{-1} was observed after exposure to ≥ 200 µg 4tPP/L and 50 ng TR/L. An increase in the number of eggs/female*d^{-1} was determined at 25 and 50 μg 4tPP/L. In F_1 the Vtg-concentration was significantly elevated in both sexes after exposure to 100 μg 4tPP/L. In addition there was a masculinization in the sex-ratio exposed to 25 ng TR/L. An increase in the number of eggs/female*d^{-1} was observed at 25 ng TR/L and a decrease was determined at 25 μg 4tPP/L. The remaining concentrations tested in F_2 revealed no adverse effects.

Wildlife ecotoxicology: from acute toxicity to low level, chronic exposure related effects (P)

TU036

Linking predator exposure and patterns of treatments with anticoagulant rodenticides by using faeces

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Rodent predators are largely exposed to anticoagulant rodenticides (ARs). To mitigate their exposure, drivers of transfer should be better characterized. The measurement of ARs residues in faeces appears as a potential non-invasive indicator to assess the exposure of vole predators. However it is unknown whether ARs residues in faeces could be correlated to treatment patterns. In 2011, fox-like faeces were sampled in 2 contexts of ARs usage, "plant protection product (PPP)/biocide" or "biocide only". PPP treatments using bromadiolone were carried out to control water vole *Arvicola terrestris* populations. PPP treatments were quantified and geographically located. In each usage category, 160 faeces of vole predators were geo-referenced and then stored at

TU037

Anticoagulant Resistance: Using metabolic inhibitors as tools for increasing efficacy

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Rodents have been noteworthy pests in agricultural areas for decades. Because of their persistence in diverse ecosystems anticoagulant rodenticides have been heavily used throughout the world. This continued use has led to the development of resistance to anticoagulant rodenticides. To investigate the potential mechanisms involved in the development of anticoagulant resistance, liver microsome preparations were made from anticoagulant exposed and unexposed voles. Using *in-vitro* microsome incubations it was determined that exposed females voles metabolize 57.8% and 25.1% more diphacinone and chlorophacinone than unexposed animals. The same trend is true in males where exposed animals metabolized 27.5% and 13.1% more diphacinone and chlorophacinone respectively. Using these data metabolic inhibitors were added to microsome incubations to determine if anticoagulant metabolism could be reduced in preparations from exposed animal livers and therefore represent a potential target for improved rodenticide bait efficacy. The addition of two types of fruit juice inhibited metabolism of chlorophacinone up to 82% in microsome incubations. Since this inhibition could translate into an increase in efficacy of baits, a taste preference trial was performed to determine if voles would have an aversion to baits containing the fruit juice. In live animal caged trials, voles showed no taste preference or aversion to baits containing up to 25% juice. These studies represent novel work into the reformulation of anticoagulant baits to address decreased efficacy as a result of the development of resistance. This increase in bait efficacy could result in a reduction in the amount of anticoagulant rodenticides needed to control rodent populations and therefore the environmental impact of these actions. Further work needs to be done to determine if the *in-vitro* metabolic inhibition will translate into an increase in efficacy.

TU038

Exposure of nestlings Red Kite to rodenticides, PAHs and metals

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The red kite *Milvus milvus* has experienced a population decline in Europe and is consequently classed as “Near Threatened” by the International Union for Conservation of Nature. Poisoning by pesticides, notably anticholinesterase chemicals and anticoagulant rodenticides, is considered one of the primary threats to this raptor. Moreover, some studies have suggested that it can be exposed to other chemicals such as metals and/or (non-pesticides) POPs but this remains weakly documented. In the framework of the French national conservation plan, some breeding populations of red kite are monitored in France. Thus, the breeding success is assessed for numerous pairs of kites and several tens to hundreds of nestlings are handled for biometric assessment, ringing, and wing tagging each year since 2006. Given the threat that chemicals may represent for red kites, this offers an opportunity for blood sampling in order to assess exposure to chemicals. During the spring 2013, a small quantity of blood (~ 1.5 ml) was sampled from 130 nestling kites in the main breeding populations of France in Corsica, Auvergne, and Franche-Comté regions. Blood residues of anticoagulant rodenticides (AVKs), metals and polycyclic aromatic hydrocarbons (PAHs) are currently being measured. Eight unhatched eggs were also collected and eggshell thickness and chemical residues will be also measured. The results we expect to present at the congress will focus on the relationships between chemical exposure and body conditions of nestlings or the breeding success between and within the monitored sub-populations. This study is partly funded by the Agence Nationale de la Recherche (convention 2009CESA00801) in the framework of the RODENT programme and the French ministry for Ecology and Sustainable Development in the framework of the national plan for the conservation of Red Kite.

TU039

Differences in residues of anticoagulant rodenticides among species and sub-populations of predators

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Anticoagulant rodenticides (AR) are substances used as pesticides for rodent control. These have an inhibitory action on the vitamin K epoxide reductase, responsible for recycling the vitamin K required for the production and activation of clotting factors II, VII, IX and X. The second generation (SGARs) have been found to be accumulative in non-target species and the consequences of this are still not well know. The aim of this research is to study the presence and concentration of ARs in tissues of predators potentially at risk of exposure. We analyzed ARs in liver of 148 wild animals found dead in Catalonia and the Balearic Islands (NE Spain). The species included 57 mammals and 91 birds: Algerian hedgehog (*Atelerix algirus*, n =46), European hedgehog (*Erinaceus europeus*, n =11), marsh harrier (*Circus aeruginosus*, n =13), barn owl (*Tyto alba*, n =21), common buzzard (*Buteo buteo*, n =25), European scops owl (*Otus scops*, n =25) and tawny owl (*Strix aluco*, n =7). Samples were extracted with dichloromethane-acetone (70/30, v/v), purified with neutral alumina cartridges and analyzed by LC–ESI-MS. SGARs were detected in 57% of the studied animals and 14% exceeded >200 ng ΣSGARs/g in liver. This threshold level, potentially associated with lethal poisoning, was exceeded most frequently in barn owl (36%) and common buzzard (24%). The SGARS identified were difenacoum (29%), bromadiolone (28%), brodifacoum (27%), flocoumafen (14%) and difethialone (5%). In both sampling areas we found a wide spatial distribution of positive animals. A higher percentage of SGARs positives was found in the European scops owl in the Balearic Islands (79%) than in Catalonia (17%), which may be related to the sedentary presence of the island population compared to the African migratory pattern of the continental one. A lower exposure to SGARs was found in the Algerian hedgehog from the Balearic Islands (46%) than in the European hedgehog from Catalonia (82%), which may be explained by differences in diet or habitat use, because the Algerian is more insectivorous and lives in drier ecosystems. These results show a significant exposure to SGARs in all the studied predators, but it was found that the migratory pattern and diet-habitat use in sub-populations of the same species or closely-related species may be important factors to consider in the risk assessment of the use these pesticides.

TU040

Equations for lipid normalization of carbon stable isotope ratios in aquatic bird eggs

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Stable isotope ratios are biogeochemical tracers that can be used to determine the source of nutrients and contaminants in avian eggs. However, the interpretation of stable carbon ratios in lipid-rich eggs is complicated because ^{13}C is depleted in lipids. Variation in ^{13}C abundance can therefore be obscured by variation in percent lipids. Past attempts to establish an algebraic equation to correct carbon isotope ratios for lipid content in eggs have been unsuccessful, possibly because they relied partly on data from coastal or migratory species that may obtain egg lipids from different habitats than egg protein. We measured carbon, nitrogen and sulphur stable isotope ratios in 175 eggs from eight species of aquatic birds. Carbon, nitrogen and sulphur isotopes were enriched in lipid-extracted egg samples compared with non extracted egg samples. A logarithmic equation using the C:N ratio and carbon isotope ratio from the non extracted egg tissue calculated 90% of the lipid-extracted carbon isotope ratios within ±0.5‰. Calculating separate equations for eggs laid by species in different habitats (pelagic, offshore and terrestrial-influenced) improved the fit. A logarithmic equation, rather than a linear equation as often used for muscle, was necessary to accurately correct for lipid content because the relatively high lipid content of eggs compared with muscle meant that a linear relationship did not accurately approximate the relationship between percent lipids and the C:N ratio. Because lipid extraction alters sulphur and nitrogen isotope ratios (and cannot be corrected algebraically), we suggest that isotopic measurement on bulk tissue followed by algebraic lipid normalization of carbon stable isotope ratio is often a good solution for homogenated eggs, at least when it is not possible to complete separate chemical analyses for each isotope.

TU041

Is shot and ammunition a significant lead exposure pathway in predatory birds in Britain?

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Lead (Pb) is a highly toxic metal that has no known biological requirement and is released into the environment through various industrial processes and from the use of Pb ammunition and shot. There has been increasing concern over the impacts on birds of prey from ingestion of un-retrieved injured and dead game that contain Pb shot or ammunition. However, there is little recent information on the exposure of predatory birds to Pb [from any source] in Britain. In this study, we aimed to assess exposure to and likely effects of Pb in predatory and scavenging birds in Britain and use Pb isotope ratios to identify the importance of different sources. We measured Pb residues (as a proxy for exposure) in the carcasses of four species collected from throughout Britain between 2006 and 2012 as part of the Predatory Bird Monitoring Scheme (PBMS: http://pbms.ceh.ac.uk/). Two species (common buzzard *Buteo buteo*, red kite *Milvus milvus*) are scavengers and potentially at particular risk from consumption of Pb ammunition and shot. The others (Eurasian sparrowhawk *Accipiter nisus* and barn owl *Tyto alba)* prey predominantly upon live passerines or small mammals and we hypothesise are less likely to be exposed to Pb from shot and ammunition. We report how total Pb concentrations and isotope ratios vary between species and test whether (i) liver residues are significantly higher in scavenging than non-scavenging species, and (ii) if isotope ratios indicate that scavenging species are the most likely to be exposed to Pb shot and ammunition. We also compare measured liver Pb residues to concentrations associated with clinical and sub-clinical adverse effects in Falconiforme species.

TU042

Mercury in waterfowl from the Ebro delta (NE Spain): trends over time and intra-/interspecific variations

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The Ebro delta is a 320 km^2 wetland area (NE Spain) of special interest for nesting and migratory birds, but with significant environmental threats due chemical products used both in agricultural areas and in the industrial activity developed in its river basin. Therefore, wild bird populations are at risk due to the presence of toxic elements in the environment, particularly non degradable elements such as mercury. Mercury is a global pollutant of special concern because it is highly toxic and it accumulates in the tissues of wildlife species and can adversely affect reproduction. On the other hand, selenium is known to be very active at counteracting Hg toxicity. Small amounts of selenium are essential for health, but it becomes toxic at high concentrations and causes low survival rates of chicks and adults. In this context, the total mercury (Hg) and selenium (Se) concentration have been determined in liver samples from 10 species of waterfowl (n=537) hunted in the Ebro delta. This liver samples came from two different sampling periods, 1993-95 (n=102) and 2007-11 (n=435). It has been observed a significant reduction of the mercury levels in the waterfowl species in the last twenty years (GLM, $F_{1,307}$=12.897, p< 0.001). The mercury accumulation was marked by the diet of

these species, thus a major ingestion of animal prey was associated with higher levels of mercury, as observed in species such as common shoveler (*Anas clypeata*) (1,923±343 d.w., *n*=35, 2007-11 period). Pintail (*Anas acuta*) (4,996±1234, *n*=14, 2007-11 period) and common shoveler (*Anas clypeata*) (4,311±385 d.w., *n*=35, 2007-11 period) were the species with higher levels of selenium. Both mercury and selenium generally show major accumulation in adults and in female individuals. Highest Hg levels in females contrast with the opposite trend found in other families of birds; and we discuss if the capital breeder strategy of ducks may explain this difference. Finally, the relationship between both elements was studied. Significant correlations were found in some species, such us mallards (*Anas platyrhynchos*) ($r=0.602$, $p< 0.001$), but it was not significant in the common shoveler (*Anas clypeata*), despite being the species with the highest levels of mercury and selenium ($r=0.219$, $p=0.206$).

TU043

Bioaccumulation of fipronil in Magellanic penguin (Spheniscus magellanicus)

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Anthropogenic chemicals pose a potential risk to human population and wildlife health. Organochlorine insecticide fipronil, was produced to combat insects when in abundance, helping in rice farming, management of large lawns and residential pest control. It is persistent in the environment, classified as highly toxic to some animals and can cause mortality to some birds at low concentrations. Its metabolite such as fipronil sulfone is more persistent and toxic than the parent compound. Since there are few studies about its accumulation and biotransformation in the aquatic environment, this research aimed to provide insight into bioaccumulation of fipronil in the Magellanic penguin (*Spheniscus magellanicus*). Liver from adults (n=09) and juveniles (n=10) penguins either found dead on the beach or that died in the rehabilitation center in the state of Rio Grande do Sul, southern Brazil were collected for analysis. Samples were solvent extracted with hexane:ethyl acetate (1:1 v/v) followed by sonication and further analyzed on HP 6890-GC/MS. The fipronil concentrations were below the detection limit of 20 ng L^{-1} and therefore, more data are necessary to effectively assess the effects of this pesticide in seabirds and their risks and its environmental contamination. Financial support FAPESP 2010/07227-2 and 2013/05491-2.

TU044

Persistent organic pollutants in yearlings of Magellanic penguins (Spheniscus magellanicus) found on the southern and southeastern coast of Brazil.

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The Magellanic penguin, *Spheniscus magellanicus,* is the most abundant of the penguins that live in temperate regions of the Southwestern Atlantic Ocean. Its breeding season runs from October to March, when it feeds off the coast of Argentina and southern Chile. In its pelagic phase, the species migrates north and winters on the continental shelf off Uruguay and Brazil. Juvenile individuals are frequently found dead on the beaches of southern and southeastern coast of Brazil in the austral winter. Living specimens were found debilitated (*i.e*. hypothermic, cachectic and apathetic) and sent to rehabilitation centers. The present study assessed the occurrence of persistent organic pollutants (POPs) in Magellanic penguins found debilitated on the beaches of the states of Rio de Janeiro, São Paulo, Santa Catarina and Rio Grande do Sul, Brazil, between 2008 - 2011. Liver samples of fifty six yearlings were analyzed, due to the body state, as the birds were found virtually without adipose tissue. The following POPs concentrations in wet weight were found: ∑ PCBs (3.2 to 2794 ng g^{-1}), ∑ DDTs (2.3 to 275 ng g^{-1}), ∑ HCHs (1.0 to 19.8 ng g^{-1}) and HCB (2.2 to 108 ng g^{-1}). Among the PCBs, there was a predominance of hexachlorobiphenyls (138 and 153) and heptachlorobiphenyls (180 and 187). Among the organochlorinated pesticides, DDT predominated, mainly in the *p,p*'-DDE form. In a general way, the concentrations of POPs found in the specimens of *S. magellanicus* reached levels (10^2 to 10^3 ng g^{-1}) similar than those found in *Pygoscelis adelie* and *Pygoscelis papua* from Antarctica. Although penguins appeared as good biomonitors since they show the distribution and fate of POPs in the Southwestern Atlantic Ocean, ecological factors (*e.g*. accumulation and biomagnification of pollutants through the food chain) and/or physiological/biological aspects (*e.g.,* mobilization of lipids-pollutants in cachectic individuals) should also be considered.

TU045

Chronic exposures of persistent organic pollutants (POPs) and their hydroxylated metabolites in the brain of free-ranging toothed and baleen whales

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Cetaceans are chronically exposed to environmental contaminants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) through food web bioaccumulation. A part of these compounds may go through metabolic transformation into hydroxylated analogues (OH-PCBs and OH-PBDEs), and besides, OH-PBDEs are known to be biosynthesized by marine algae and cyanobacteria symbiotic with marine sponges. Some of OH-PCBs and OH-PBDEs congeners structurally resemble thyroid hormones (T_4 and T_3) which are essential for normal brain development. Over the past decade, *in vitro* and *in vivo* studies on rodents and human epidemiological studies revealed the link between the exposure of OH-PCBs and/or OH-PBDEs and effects on the brain function and development. The results included suppressed gene transcription and cell death, disruption of neuron development, hearing loss and deficits in learning and memory in rats and lower IQ and neurocognitive function in children. However, there are only few reports on the levels of the hydroxylated metabolites in the cetacean brains. In this study, residue levels and patterns of PCBs, PBDEs, OH-PCBs and OH-PBDEs in the blood and brain of seven species of free-ranging toothed and baleen whales, including finless porpoises (*Neophocaena phocaenoides*, $n = 15$), striped dolphins (*Stenella coeruleoalba*, $n = 5$), melon-headed whales (*Peponocephala electra*, $n = 5$), killer whales (*Orcinus orca*, $n = 2$), Dall's porpoises (*Phocoenoides dalli*, $n = 2$), a minke whale (*Balaenoptera acutorostrata*, $n = 1$) and a fin whale (*Balaenoptera physalus*, $n = 1$) stranded or by-caught along Japanese coasts, were determined. PCBs, PBDEs, OH-PCBs and OH-PBDEs were detected from the blood and brain of all the cetacean species analyzed. Levels of the compounds in the blood and brain had positive correlations among each other ($p < 0.05$), indicating transfer of all these compounds through the blood-brain barrier. Further, the levels in the brain were compared to the laboratory determined effect threshold levels. OH-PCBs levels in the majority of toothed whales have exceeded the *in vitro* suppression level of thyroid hormone mediated gene transcription in rat cerebellar cells, with much higher level in the brain of a neonate killer whale (3600 pg/g wet wt.). These results indicate possible adverse effects of these compounds on cetaceans, and further studies are needed for the risk assessment of wild marine mammals.

TU046

Associations between the anogenital distance and environmental concentrations of perfluoroalkyl acids and DDE in wild male mink

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The anogenital distance (AGD) is an established measurement for assessing foetal exposure to endocrine-disrupting chemicals in rodents and humans. By studying wild male mink (*Neovison vison*), possible associations between the anogenital distance and concentrations of perfluoralkyl acids (PFAAs) and p'p-dichloro-diphenyl-dichloroethylene (DDE) were investigated. The wild mink has been acknowledged as a suitable sentinel species for environmental pollution as it is a semi-aquatic top predator. In addition, the home range of the mink is relatively small, suggesting that the pollution within and in the vicinity of the home range is reflected in the mink. Wild mink (n=101) were collected in four areas in Sweden. Concentrations of perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluoroalkyl carboxylates (C8-C13) were analyzed in liver and summarized for each mink (∑PFAAs). DDE was analysed in subcutaneous fat. The distance from the anus to the preputial opening (AGD) was measured and a weight-normalized index calculated [AGI =AGD/weight (mm/kg)]. Two multiple regression models, including age and body condition, were used to analyze the influence of ∑PFAAs and DDE on AGI (respectively), using log-transformed data and sample area as random factor. Significant negative linear relationships between AGI and ∑PFAAs or DDE were found ($p=0.02$ for both). Excluding one outlier, the range of the anogenital index was 46 to 84 mm/kg (mean 63 mm/kg and SD 8). The range of the

concentrations of DDE was 8.8-9710 ng/g lipid weight (mean 480 ng/g and SD 1100).High concentrations of ∑PFAAs were found (mean 1370 ng/g wet weight and range 29.1- 22 000 ng/g). PFAAs have been associated with decreased serum testosterone levels in various species (Biegel et al 1995, Shi et al 2007, Joensen et al 2013) and DDE is a known anti-androgen (Kelce et al 1995). This study suggests that chronic exposure to ∑PFAA and DDE at levels found in the environment in Sweden is associated with anti-androgenic effects in wild male mink.

TU047

Long-term environmental exposure to persistent organic pollutants in a municipal landfill, and their effects on the breeding success in European starlings (Sturnus vulgaris)

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The Chemical Management Plan (CMP) was established in 2006 by the Government of Canada to provide funding for research and monitoring of chemicals that pose a potential threat to human and environmental health. A terrestrial component of this program was set up in 2008 to monitor contaminant levels in European starlings (*Sturnus vulgaris*) in five urban centers across Canada. Although some of the highest PBDE levels in North America were identified in the Delta municipal landfill, situated outside of Vancouver, British Columbia, no research was established to identify the effects of these contaminants on the breeding performance of the starlings. In this study, we compared the breeding success of Delta starlings to the breeding success of starlings at two rural reference sites. As starlings are able to double brood, breeding success was established for both a peak nesting period, and a secondary nesting period. Measured ΣPBDEs, ΣPCBs, and select OCs in the eggs of starlings breeding at Delta were significantly higher than those measured in the Glen Valley reference site, with PBDEs being the highest (217.85 ± 8.14 µg/kg ww). Overall breeding success was lowest at Delta in the peak breeding season with fewer successful nests, significantly smaller brood sizes at hatch, significantly fewer fledglings, and significantly smaller chicks. In both breeding periods, Delta also had significantly smaller eggs than both reference sites. Behaviourally, Delta starlings provisioned significantly less for their chicks, and provided poorer quality food items, including human refuse. Contaminant analysis of the food items at Delta also revealed that the human refuse was the largest source of PBDEs. Based on our observations, we suggest that long-term environmental exposure to contaminants in Delta has a physiological effect on the breeding success in these birds.

TU048

Levels of organochlorine compounds in eggs of gull-billed terns and assessment of eggshell pigments as potential biomarkers

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Gull-billed tern (*Gelochelidon nilotica*) can be exposed to persistent organic pollutants in the breeding grounds in Europe, but also in the wintering quarters in Africa. This tern species is particularly different to the rest of terns because it uses more terrestrial habitats and feeds mostly on terrestrial insects. The levels of organochlorine pesticides and polychlorinated biphenyls (PCBs) were determined by GC-ECD in 97 eggs of gull-billed terns collected after the abandonment of a colony in the Marshes of Mesas de Asta (Cadiz, S Spain). The main objective of this work was to determine the relationships between the levels organochlorine compounds in the egg content and the embryo development, egg biometrics and eggshell pigmentation. Porphyrins and biliverdin were measured in eggshells by HPLC-UV-Vis and its use as potential biomarkers of the exposure to organochlorine contaminants was discussed. Most of the eggs (77%) were embryonated and the embryonic stage was positively correlated with the weight of the content ($r=0.272$, $p=0.007$) and negatively with the shell index ($r=-0.515$, $p< 0.001$). The most abundant organochlorine was p,p'-DDE (11 μg/g lipid weight, l.w.; 3.8 μg/g wet weight), with comparatively higher concentrations that other populations of gull-billed terns or other terns species in Spain. Also relevant, but at much lower levels, were the concentrations of ΣPCBs (1.3 μg/g l.w.). ΣCyclodienes levels were significantly higher in embryonated (0.05 μg/g l.w.) than in non-embryonated eggs (0.03 μg/g l.w.; $p=0.012$), and this trend was also observed for other organochlorines detected in these samples and for the calculations made for the egg burden (all $p\leq0.013$). The primary pigment in the eggshell was protoporphyrin IX (a precursor of heme group), followed biliverdin (a breakdown product of heme group). The concentrations of both pigments were highly correlated ($r=0.901$, $p< 0.001$). It has also been observed that biliverdin was in greater concentration in the eggshells of embryonated eggs ($p=0.045$). Moreover, biliverdin level in the eggshell was negatively correlated with the concentration of p, p' -DDE in the content, even if we consider the effect the embryo development in the analysis ($p=0.023$). This result indicates that the concentration of biliverdin in eggshell could be used as a non-invasive effect biomarker of organochlorine contaminants.

TU049

Biomarkers of exposure and effects to detect the toxic effects in wood mice along a gradient of pollution

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Multiple biomarkers of exposure (bioaccumulation of metals) and effect (body condition index, histopathology, and hematological parameters) were quantified to assess the adverse effects induced by trace metals (TMs) from cellular to individual levels in wild wood mouse (*Apodemus sylvaticus*). Wood mice were collected along a gradient of Cd, Pb, and Zn pollution around the former smelter of Metaleurop (northern France) in activity from 1896 to 2003. Concentrations of Cd and Pb were measured in the liver, kidneys and hair. Health status based on thresholds of toxicity derived from bioaccumulation levels for single metal exposure (LOAELs) was defined for each specimen. Individuals exhibiting one or more internal concentrations above these levels were considered "at risk" for metal-induced stress. Histological alterations specific to TMs were observed in the liver and in kidneys. Hematocrit, leukocytes and granulated erythrocytes levels were measured on blood samples. The body condition index was calculated from the body length and body mass. Internal Pb concentrations in the organs and in hair increased along the gradient of pollution while Cd accumulation in organs exhibited a bell-shaped curve (highest Cd levels were observed in the individuals trapped on the moderately polluted site). Necrosis in liver and tubular dilatation in kidneys significantly increased with Cd or Pb concentrations in soils or in organs indicating that individuals living around Metaleurop smelter exhibited tissular lesions due to Cd and Pb contamination. Body condition was not significantly related to any of the other biomarkers studied. Hematocrit level and leukocytes number decreased with Cd concentrations in the organs. Variation of those hematological parameters was interpreted as a warning for potential negative effects of Cd exposure on the oxygen transport capacity of blood (*e.g*. anemia). The individuals considered at risk for metal-induced stress exhibited higher severity of histological alterations and tended to have lower hematocrit levels than other individuals. The different biomarkers assessed in the present study can be considered as suitable for metal induced stress in wild wood mice. Their combined use showed interesting results and the possibility of observing multiple adverse effects at different levels of organization. For both scientific and ethic reasons, further studies should evaluate non lethal biomarkers to promote them in ecotoxicology.

TU050

Acute toxicity and genotoxic effects of formaldehyde to tadpoles of Lithobates catesbeianus (american bullfrog)

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Amphibians are an useful bioindicator of environmental disturbance because of their intensive interaction with all constituents of biosphere: air, soil and water. Several indicators, such as density decreasing of population, mutations and somatic malformations have been described in amphibians as consequence of pollution in different environmental exposures. The worldwide frog *Lithobates catesbeianus* (american bullfrog) is a suitable sentinel species generally applied in studies of ecological hazard assessment, specially when the focus is the monitoring of pollution effects on freshwater. The occurrence of micronuclei (MN) in erythrocytes of the peripheral blood of such animals has been used over the past years, as a biomarker for testing genotoxic effects on aquatic organisms exposed to environmental contaminants. Formaldehyde is a disinfectant used on a large scale of domestic and industrial proposition, and it is the main active ingredient of many formulations of sanitizing. It can be easily absorbed by aquatic animals due to its high water solubility. The aim of the present study was to determine the $CL_{50\text{-}96h}$ of formaldehyde to tadpoles of *L. catesbeianus* as well as observe the genotoxic effects caused by assessing its mutagenic potential in inducing the formation of micronuclei (MN). The acute toxicity test were carried out during 96 hours of exposure time. The experiment was run according to the static renewal method, with five concentration-test of 6, 9, 12, 15, and 18 mg/L of formaldehyde, plus a control group. Eight tadpoles were exposed at each concentration, which were set under three simultaneous replicates. The result of $CL_{50\text{-}96h}$ of 10.53 mg/L of formaldehyde was calculated by the Trimmed Spearman Karber Test. In order to perform the MN test, peripheral blood samples of each survivor organism were collected, at the end of exposure time, by the puncturing of the iliac tail vein. Afterwards, the slides were stained by the Fuelgen / Fast-Green method and analyzed in blind test (2.000 erythrocytes / organism). As a result it was noticed a tendency of dose-dependent induction of micronuclei formation, with an increase of 1.47 MN to every 1 mg / L of test-solution (test conducted by Poisson regression). It was possible to conclude that concentrations of formaldehyde tested

in the present study can cause high mortalities to the tadpoles as well as lead survivor population to suffer genotoxic effects.

TU051

Cytogenetic damage progression in peripheral erythrocytes of fish (Anguilla anguilla) upon cessation of exposure to a deltamethrin-based insecticide

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Pyrethroids are among the most used insecticides worldwide and considered one of the most toxic groups to fish. Deltamethrin, the active ingredient of the commercial formulation Decis®, is a synthetic pyrethroid with insecticidal properties, effective against a multiplicity of pests. The occurrence of deltamethrin in the aquatic environment is well-established, but in what concerns to the possible genotoxic effects of Decis® in non-target organisms, namely fish, they remain largely unknown. Hence, the main goal of this work was to evaluate the cytogenetic damaging potential of Decis® in European eel (*Anguilla anguilla*), adopting the erythrocytic nuclear abnormalities (ENAs) assay. Moreover, it was intended to investigate the damage progression upon cessation of insecticide exposure. In order to provide indirect information on the erythrocyte catabolism and erythropoiesis rate, as a measure of hematological dynamics, the frequency of immature erythrocytes (IE) was also determined. Thus, fish were exposed to 17.5 and 35 μgL^{-1} of Decis® (equivalent to 0.05 and 0.1 μgL^{-1} of deltamethrin, respectively) during 1 and 3 days. Thereafter, fish were transferred to clean water and kept during 1, 7 and 14 days, in order to evaluate an eventual recovery. The analysis of results demonstrated a clear potential to induce chromosomal damage following the third day of exposure, depicted in an ENA frequency increase for both tested concentrations. The transient nature of this particular kind of damage (cytogenetic) was also demonstrated, since ENA frequency returned to the control levels 1 and 7 days after cessation of the exposure, respectively for the higher and the lower Decis® concentrations. This response pattern provided evidence towards a rapid metabolization and elimination of the constituents of the tested formulation by *A. anguilla*. In addition, the IE results suggested an unaltered erythropoiesis rate during the entire experiment, with the exception of day 14 post-exposure (where IE frequency was increased). Consequently, a dilution effect resulting from an erythropoiesis increment should be excluded as a determinant factor affecting ENA frequency in the first 7 days of the post-exposure period. Overall, the demonstrated genotoxic properties of Decis® pointed out increased risk factors to fish.

TU052

Feral finfish, and their relationships with sediments and seawater, as a tool for risk assessment of PAHs in chronically polluted environments.

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An integrated study has been carried out of the fate and effects of PAHs in fish living in a chronically polluted environment. Total PAH concentrations in different target organs (muscle, liver and gills), have been determined in five species of feral fish and possible histopathological effects and correlations of all these values with concentrations found in sediments and water column have been studied in two of these species. The Biota-to-sediment accumulation factors (BSAFs) and the Toxic Potency Assessment (TEQ) of sediment for fish have been calculated. Results show that levels found in target organs and the TEQs of sediment calculated for fish are of special concern. However, the index of pathologies shows a relatively low impact of PAHs on fish health. The use of feral finfish in risk assessment for PAHs in chronically polluted environments has been proved to be a useful tool to complement environmental diagnoses and improve their accuracy. This approach combines the measurement of total concentrations in different target organs of several appropriate species, the study of histopathological effects, and correlations between all these results and the concentrations found in associated sediments and column water.

TU053

Triclosan interferes with the thyroid axis in Sheepshead minnows (Cyprinodon variegatus)

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The sheepshead minnow is widely used in ecotoxicological studies and such investigations have begun to focus on potential disruption of the thyroid axis. A previous study established developmental patterns of thyroid hormones levels during the development of sheepshead minnow from embryo to juvenile and adults. The levels peaked around day 12 post hatching, which coincides with the larvae to juvenile transition of this species. We evaluated if the ecotoxicological effects of thyroid disruptors will be most significant during this period of early developmental processes, where thyroid hormones play a prominent role. Therefore we established an *in vivo* triclosan (TCS) exposition protocol of eggs and larvae till day 12 post hatching. Couples of three females and two males were placed in breeding chambers designed for this experiment. Eggs were collected and maintained in seawater. Embryos were selected under a dissection microscope, randomly assigned to each of five treatment groups: Control, DMSO control, 20 µg/L TCS, 50 µg/L TCS and 100 µg/L TCS and placed in incubation dishes (50 per dish) at 25°C. On day 6, embryos hatched and larvae were transferred to 1L dishes. The larvae were fed on artemias and on flaked fish food till day 12 post hatching when the fish were sampled. The pooled samples were taken from several incubation dishes and divided in three replicate batches of 10 individuals. Enzyme-linked immunoassay were used and validated for analysis of T4 and T3 after extraction from whole fish. From each exposition group, 5 individuals were placed in formalin fixative for histology. Length and body mass were measured. Hatching success, gross in vivo observations, thyroid hormone levels and histology data will be determined and discussed. This study demonstrates that TCS acts on the fish thyroid axis. The fact that TCS is commonly detected in aquatic ecosystems, and the importance of the thyroid in basic physiological processes such as metabolism and nervous tissue development means that interference of TCS with this axis may have profound consequences for organism health and survival of aquatic wildlife. The results of the present study highlight the need for more detailed studies of the effects of TCS, which accumulates in sediments and organisms in aquatic environments.

TU054

Are Ionic Liquids Green Solvents?

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One of the most important targets of green chemistry is the use of renewable raw materials because materials such as natural gas, coal or petroleum are irrevocably decreasing. A green alternative to these compounds are the ionic liquids (IL_s), which have been studied as possible substitute in some scientific and industry applications. These chemicals are organic salts melt at room temperature. Their unique properties, possibilities of combination possibilities, low volatility and flammability and insignificant vapour pressure, make them as a strong option to be considered as green solvents.[1,2] Nowadays there is a huge interest about these compounds and scientists are publishing a lot of articles related to their physicochemical properties and applications. However, there is a lack of studies about their ecotoxicology assessment. With the aim of completing this information, we present several ecotoxicological studies of the following ionic liquids: 1-butylpyridinium tetrafluoroborate ([bpy][BF_4]), 1-propylpyridinium tetrafluoroborate ([ppy][BF_4]),1-butyl-2-methylpyridinium tetrafluorobarate ([B2]), 1-butyl-3-methylpyridinium tetrafluoroborate([B3]) and 1-butyl-4-methylpyridinium tetrafluoroborate ([B4]). The tests used in this research work are the inhibition of the bioluminescence using *Vibrio fischeri* (UNE-EN-ISO 1348-3) and immobilization test with *Daphnia magna* (OECD 202). Additionally, interesting relationships between ecotoxicity and structure of the studied chemicals have also been obtained. [3] Moreover, according to the physicochemical properties of the ILs, partition coefficients of the compounds have been calculated using two different methodologies: ALOGPs and Kowwin2. Finally, the variation of the EC_{50} regarding the log P has also been studied. Key words: ionic liquids, green solvents, ecotoxicity, EC_{50}. [1] Pinto P.C.A.G, Costa S.P.F., Lima J.L.F.C, Saraiva M.L.M.F.S. Ecotoxicology and Environmental Safety, **2012,** 80, 97-102. [2] Pham T.P.T., Cho C.W., Yun Y.S. Water Research, **2010**. 44, 352-372. [3]Jastorff B., Störmann R., Ranke J., Mölter K., Stock F., Oberheitmann B., Hoffmann W., Hoffmann J., Nüchter M., Ondruschka B., Filser J. Green Chemistry. **2003**. 5,136-142.

TU055

Effects of two PBDE congeners on the moulting enzymes of the freshwater amphipod Gammarus pulex.

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Polybrominated diphenyl ethers (PBDEs) constitute a class of chemical compounds included to the composition of usual products such asplastics, textiles or electrical equipment, due to their flame retardants properties. Since 2004, PBDEs were banned in Europe and listed as Priority Substances within the European Union Water Frame Work Directive. Unfortunately, the release of PBDEs always occurs in ecosystems due to their presence in products currently in use and new products manufactured using recycled PBDE-containing material. However, only few studies have investigated their impacts on freshwater invertebrates. This work aimed to study the effects of BDE-47 and BDE-99 congeners on the chitobiase and chitinolytic enzymes activities of the freshwater amphipod *Gammarus pulex*, according to gender, PBDE concentration and time of exposure. In addition, the bioaccumulation of BDE-47 and BDE-99 were measured. The results revealed that there was a dose-response relationship for the PBDE accumulation in *G. pulex*, whatever the gender and the PBDE congeners. Female *G. pulex* bioaccumulated more PBDE than males, and especially BDE-99 which was more accumulated than BDE-47. Moreover, PBDE exposures for 96hrs have caused the inhibition of chitobiase and chitinolytic enzymes activities. This study not only indicate the importance of taking into account various confounding factors (gender, congeners, concentration) to understand the PBDE effects, but underline also disruptions of enzymes activities involved in the molting process. These disturbances suggest effects on the gammarid development and reproduction, and consequently on the gammarid population.

TU056

TOXIC EFFECTS OF 6 METALS IN FRESHWATER ORGANISMS OF DIFFERENT THOPIC LEVELS

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The Cd, Cr, Cu, Mn, Ni and Pb metals are found in high concentrations in some aquatic systems in the valley of Mexico, due to the contribution of untreated wastewater, coming from industrial, domestic and agricultural activity. The effects of these compounds on aquatic organisms are: acute exposures death. In sublethal exposures: changes at the biochemical level, as inhibition of enzyme activities, in addition to alterations in feeding, growth and development. The studies on the effect of these pollutants on native organisms are scarce and its sequelae are not known in many species in subtropical and tropical climates. In this work an evaluation of the deleterious effect of the metals Cadmium, Chromium, Copper, Manganese, Nickel and Lead was carried out on organisms belonging to different trophic levels: Microalgae *Selenastrum capricorn*atum and *Monorraphidium* sp., the copepod *Acanthocyclops*, the cladocerans *Daphnia magna*, *D. exilis* and *D. pulex*, the ostracod *Cyipris* sp. and the charal fry *Chirostoma jordani*. Static bioassays were conducted. The organisms were exposed to 5 concentrations of each metal in triplicate plus a control without toxic. The tests lasted 48 to 72 hours to determine the LC_{50} (lethal concentration 50). Also a comparison of the LC_{50} obtained was performed to compare the sensitivity of the species. The results indicated that the most sensitive organisms to metals were cladocerans and fry the charal. The toxicity of metals based on the LC_{50} was calculated (high to low toxicity): For microalgae: Cu > Cr > Cd > Ni > Mn > Pb. For copepod: Cd > Cu > Cr > Ni > Pb > Mn. For *Daphnia magna*: Cu > Cd > Cr > Pb > Ni > Mn. For *Daphnia exilis*: Cu > Cd > Ni > Cr > Pb > Mn. For *Daphnia pulex*: Cu > Cr > Cd > Ni > Pb > Mn. For the ostracod: Cd > Cu > Cr > Pb > Mn > Ni and to the fry of *Ch. jordani* Cu > Cd > Ni > Pb > Mn > Cr . Because the LC_{50} values for Cd, Cu, Cr, Ni metals are lower than those established in NOM 001-SEMARNAT for wastewater discharges in aquatic systems, it is important to continue research and monitoring to detect responses indicating the possible damage to the populations of these organisms by the action of the discharges and tensioners, to prevent irreversible deterioration of the populations in the medium and long term.

TU057

Management of the industrial biofouler Corbicula fluminea - alternative roles for old (Eco)toxicological methods

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Corbicula fluminea, commonly known as the Asian clam, is an invasive freshwater macrofouler largely spread worldwide, and inducing serious negative impacts in invaded ecosystems and infested freshwater-dependent industries.

(Eco)toxicological techniques are a powerful constituent of the toolbox supporting the development of management strategies for macrofoulers such as the Asian clam, although their establishment on the basis of successful practice rather than systematic assessment has been the most common approach. The present communication aims specifically at exploring on two fundamental avenues through which these tools can be explored: (i) the development of improved control methods using different testing frameworks; (ii) the assessment of the biofouler potential as a bioremediator of contaminated effluents. A comprehensive group of candidate control chemicals - that have been already used to control other bivalve biofoulers and/or are used within the production process of affected industries and may hence assume a dual function – were tested against the target pest and non-target environmental indicators. Long-term treatment provides the best perspectives for the control of the pest as both efficiency and environmental safety of the method are considered. The use of particular traits of the species ecophysiology, such as, in this case, sensitivity to depressed oxygen conditions may constitute a breakthrough for increasing the efficiency of particular chemical control strategies. As well, the use of biochemical biomarkers to run comprehensive studies on the variation in the efficiency of binary combinations of control chemicals can arise as a successful methodological model for the purpose. The suggested potential of the Asian clam as environmental remediator was experimentally confirmed using two metal-bearing effluents; although this represents good perspectives for an alternative use to the pest able to offset its economically damaging impacts, further studies are needed towards refinement of the configuration and maintenance of a potential bioremediation system based on filtration by the Asian clam. The presentation of this framework hopefully will stimulate discussion on alternative applications for traditional (eco)toxicological methods.

TU058

Using bioturbation as a response in sublethal toxicological assessments with L. plumulosus, N. arenaceodentata and M. mercenaria

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Most toxicity tests of benthic fauna determine the contaminant concentration that causes mortality. Although mortality is a convenient endpoint for measuring toxicity, it does not accurately capture the environmental risk in a natural environment because biota in natural systems are most often chronically, and not acutely exposed to contamination. Sublethal tests offer a different insight into the environmental impacts of pollution. Most commonly chronic toxicity tests are based on growth and reproduction or bioaccumulation; less frequently adult emergence, egg hatching, or enzyme inhibition are used. These tests typically require long experimental duration and/or sophisticated laboratory handling. Bioturbation (biological particle and solute mixing) has been used in toxicological assessment only rarely, even though studies have shown its sensitivity to sediment contamination at concentrations up to 5 orders of magnitude lower than those required by 96-h LC50 tests. In this study we examined bioturbation by benthic macroinvertebrates as a measure of sublethal sediment toxicity. We quantified particle mixing and solute transport using ^{137}Cs and ^{22}Na radiotracers, respectively. Four bioturbation parameters were calculated from time series of non-destructive vertical profiles of radiotracers in laboratory microcosms containing organisms and sediments. We compared bioturbation parameters in clean and contaminated (New Bedford Harbor Superfund Site) sediments for three species of marine and estuarine benthos commonly used in toxicological assessments: the selective deposit feeding amphipod *Leptocheirus plumulosus*, the bulk-sediment ingesting polychaete *Neanthes arenaceodentata*, and the filter-feeding bivalve *Mercenaria mercenaria*. These organisms exhibit different burrowing and feeding habits and their selection was aimed at revealing functional group dependence of contamination-induced bioturbation. Our study showed that bioturbation can be used as a sensitive and easy measure of the response of biota to sediment toxicity at sublethal contaminant doses. Furthermore, different mixing parameters are affected by contamination to a different extent. For example, compared to the control for *L. plumulosus* sediment burial rates in contaminated sediment decreased by a factor of 2, particle biodiffusion rates decreased by a factor of 10, and solute exchange showed no significant difference at the 0.10 level.

TU059

Toxic and endocrine disrupting effects of Bisphenol A on freshwater isopod Asellus aquaticus

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Bisphenol A (BPA) is an organic compound, used worldwide in the production of polycarbonate plastic and epoxy resins. It is a common chemical entering the environment via the wastewaters, and landfill leachate. BPA is not only an organic pollutant toxic to wildlife and humans, but also a well known endocrine disrupting compound (EDC), which causes disruption in the endocrine system of exposed organisms, in low environmentally relevant concentrations. It is generally recognized that BPA can seriously affect local wildlife populations in reducing survival, causing high incidence of deformities, disruption of embryogenesis in fish, amphibians, crustacean and other aquatic species. In the aquatic environment organisms can come in contact with BPA by its uptake via gills and the skin or from dietary sources via the gastrointestinal tract. The aim of our study was to determine the toxic and endocrine disrupting effects of BPA on different life stages of freshwater isopod *Asellus aquaticus* (L.), and to compare the efficiency of BPA uptake routes from water and/or food, and identify the dominant one. Conducted were 96 hour acute toxicity tests, where laboratory bred, juvenile and adult, specimens of *A. aquaticus* were exposed to BPA in water medium. The results show that juvenile organisms are more sensitive to toxic effects of BPA, with a 96 h LC_{50} of 5.7 mg L^{-1} BPA in comparison to adult rganisms with a 96 h LC_{50} of 24.5 mg L^{-1} BPA. Chronic toxicity tests were performed with juvenile and adult organisms, exposed to BPA dissolved in water or spiked on conditioned alder (*Alnus glutinosa*) leave discs, for 21 days. Toxicity endpoints were survival, growth, feeding rate, pigmentation, mobility and paralysis. The results imply, that BPA uptake from food is more efficient than uptake from water, since the toxic effects were observed at

lower BPA concentrations. Pigmentation, growth and feeding rate were proven to be most sensitive toxicity endpoints. The effects of BPA as an endocrine disruptor were investigated in short as well as long term tests, through the inhibition in moulting frequency. The effects were best noted in exposed juvenile specimens of *Asellus aquaticus*. In conclusion, our research indicates that juvenile organisms are more susceptible to lower concentrations of BPA in comparison to adults, and that food can present an important uptake route of contaminants into test species, as they are exposed to higher concentrations than in overlaying water.

TU060

Metallothionein induction by an essential metal (Zn) in a freshwater decapod crustacean of South America, Palaemonetes argentinus

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Aquatic invertebrates take up and accumulate trace metals even when both, essential and non-essential, have the potential to cause toxic effects. The aim of this study was to investigate the potential use of metallothioneins (MTs) as biomarkers of metal contamination in native shrimp *Palaemonetes argentinus,* a species of ecologic interest because of its wide distribution in different country of South America. Organisms have been exposed at different environmental concentrations of Zinc ($ZnSO_4.7H_2O$): controls (not metal exposed), shrimps exposed to 5 μg Zn L^{-1} (7.65 x10^{-5} mM), 50 μg Zn L^{-1} (7.65 x10^{-4} mM) and 500 μg Zn L^{-1} (7.65 x10^{-3} mM). A significant Zn accumulation has been observed in different body sectors. In cephalothorax, at 500 μg Zn L^{-1} (369.14 ± 49.75 μg Zn g^{-1}dw, p< 0.0001) and 50 μg Zn L^{-1} (216.65 ± 65.99 μg Zn g^{-1}dw, p< 0.01) the concentration of metal significantly increased compared to other conditions (Control= 147.08 ± 21.50 μg Zn g^{-1}dw; 5 μg Zn L^{-1}=144.74 ± 22.54 μg Zn g^{-1}dw). Greater accumulation occurred in cephalothorax compared to abdomen, especially at higher exposure concentration (500 μg Zn L^{-1}). Subcellular metal distribution was different in cephalothorax and abdomen. In cephalothorax, Zn was equally distributed between the soluble and the insoluble fractions whereas in abdomen, when total Zn increased, insoluble metal increased more markedly than the soluble one. Cytosolic levels increased greater in the cephalothorax than in the abdomen of shrimps exposed to 500 μg Zn L^{-1} compared to control. Cephalothorax of organisms exposed to the greater (500 μg Zn L^{-1}) concentration of Zn showed a significant increase in MT levels (0.651 ± 0.025 mg MTs g^{-1}ww) compared to cephalothorax from control and 5 μg Zn L^{-1} organisms (0.518 ± 0.045 mg MTs g^{-1}ww and 0.516 ± 0.085 mg MT g^{-1}ww respectively, p< 0.05). Conversely, no induction of MTs was observed in the abdomen of *P. argentinus* even at the highest exposure concentration (p>0.05). A positive and significant correlation (r^2=0.52, p< 0.01) has been observed between cephalothorax MTs and cytosolic Zn concentrations. Our results showed a fast response (96 h) of MTs induction in the cephalotorax of *P. argentinus* at environmental Zn concentrations, indicating its potential use as a biomarker of metal exposure in this native species.

TU061

Improved control of the biofouler Corbicula fluminea: combined action of chemicals and dissolved oxygen reduction

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The Asian clam *Corbicula fluminea* is amongst the major invasive species in freshwater ecosystems worldwide, causing great adverse ecological and economic impacts in natural and industrial systems. In man-made structures the damaging effects of this pest is mainly related to its biofouling activity. As the methods currently available to control the species present cost-effectiveness and environmental concerns, it is crucial to maintain the research efforts into the search for improved mitigation approaches. In this study, the possibility of enhancing the clam's susceptibility to chemical control agents by combining them with hypoxic conditions was investigated. A range of candidate chemicals with distinct toxicity mechanisms were selected for analysis in order to address the effect of this factor on the performance of the combination. Exposure of adult clams to the molluscicide niclosamide, the flocculant Polydiallyldimethylammonium chloride (polyDADMAC), ammonium nitrate, copper sulfate, potassium chloride and dimethoate under normoxic (> 7 mg L^{-1} dissolved O_2) and hypoxic (< 2 mg L^{-1} dissolved O_2) conditions was carried out. The results of the study suggest that hypoxia generally increases the efficiency of these chemicals. Such enhancing effect was found to be greater for dimethoate and lower for potassium chloride, which can be interpreted in the light of their mechanism of toxicity in bivalves. While further studies are still necessary, namely addressing selectivity issues, the assessment presented in this communication provides systematic grounds to infer on the suitability of depressed oxygen conditions to assist chemical control treatment targeted at this species' both in infested industrial systems and open waters.

TU062

Nutritional stress as a confounding factor in the evaluation of DNA integrity in the freshwater mussel, Dreissena polymorpha

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The measure of DNA damage by the comet assay is a recommended biomarker to evaluate the genotoxicity of compounds or contaminated ecosystems in wildlife species. However, our knowledge on the incidence of both internal (sex, physiological status) and external (temperature, food deprivation) confounding factors on the response of genotoxicity biomarker is still scarce. The objective of the present study was to measure on a long-term laboratory exposure (5 months) the genotoxicity of a food deprivation on haemocytes of the zebra mussel, by means of the comet assay. Haemocytes which are involved in many physiological processes revealed to be sensitive cells to genotoxic stress. Mussels were fed twice a week. Three food conditions were tested: 100% (*ad libitum*-control), 10% and 0% of a mix of two microalgae (*Scenedesmus obliquus* : *Chlorella pyrenoïdosa* 50:50), at a final concentration of one million/day/mussel for the control condition. Results showed a significant increase of DNA damage in haemocytes from individuals exposed to a food deprivation (10% and 0%) compared to controls, which showed a low variability along the experiment of the baseline level of DNA damage. A greater genotoxic effect was observed in low-fed individuals (10% condition), which could be the consequence of a higher filtration rate in response to the food deprivation; whereas unfed mussels (condition 0%) were probably in a "latent state". These genotoxicity results were in accordance with the higher rate of mortality observed in low-fed organisms (10% condition). In relation with their physiological status, freshwater mussels would promote different mechanisms in response to the genotoxic stress. The transitory increase of DNA damage observed during the 1st and the 4th month of the experiment in stressed-organisms (10% and 0% conditions) decreased to a background level after to have reached a threshold level of DNA damage. This threshold revealed to be higher for low-fed individuals (condition 10%), indicating their ability to support a higher degree of DNA damage in their haemocytes before initiating DNA repair. In opposition, unfed mussels showed a trend in a higher circulating haemocytes concentration within hemolymph, probably favoring cell renewal in response to the genotoxic stress.The present study pointed out the importance to improve our knowledges on the response of genotoxicity biomarkers with a view to their use in biomonitoring studies.

TU063

Development of a Cellular Efflux Pump Inhibition Assay (CEPIA) for Lymnea stagnalis eggs.

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A combined CEPIA (Cellular Efflux Pump Inhibition Assay) and toxicity assay has been developed for analyzing mixture toxicity in the eggs of the pond snail *Lymnea stagnalis*. Efflux pumps homologous to ABCB pumps were found to be present in eggs less than 24h old. The effect on the efflux pump activity of several organic and inorganic chemicals was tested using rhodamin B and Calcein-AM as fluorescent substrates. The efflux of rhodamin B could be inhibited by verapamil in a dose-dependent manner while efflux rates were increased by several compounds including organic and inorganic mercury. Determination of viability of the eggs was easily performed by observing presence of embryonal movements using digital microscope recordings. This was used for LC_{50} determinations of the chemicals tested for modifying efflux pump activity.

TU064

Genotoxic potential of glyphosate-based herbicides to fish and elucidation of DNA damaging mechanisms - comparison between the active ingredient and the major environmental breakdown product (AMPA)

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Agriculture practices have led to pronounced adverse repercussions on the aquatic environment mainly due to the introduction of agrochemicals. In particular, herbicides are considered among the most hazardous contaminants of water bodies, since they easily reach these ecosystems. In general, studies on the effects of herbicides concerning aquatic organisms are focused on the active ingredients of the commercial formulations. Nevertheless, the assessment of the direct impact of chemicals that may occur in the environment as breakdown products of the parental compound could be considered even more ecotoxicologically relevant. Glyphosate, the active ingredient of various well-known herbicide preparations, easily reaches the aquatic compartment being naturally degraded into aminomethylphosphonic

acid (AMPA). The main goal of the present research was to compare the DNA damaging potential of glyphosate and AMPA in fish (*Anguilla anguilla*), adopting environmentally realistic concentrations. Hence, the comet assay was applied to blood cells, either as the standard procedure, or with an extra step involving DNA lesion-specific repair enzymes (FPG and EndoIII) in an attempt to identify the occurrence of oxidatively damaged DNA. Fish were exposed to glyphosate (17.9 and 35.7 μg.L^{-1}) and AMPA (11.8 and 23.6 μg.L^{-1}), as equivalent molar concentrations, during 1 and 3 days. After the first day of exposure, the standard comet assay demonstrated potential to induce DNA damage for both concentrations of glyphosate and AMPA. On the other hand, the third day of exposure showed that only the lowest concentration of glyphosate was able to induce DNA damage. Concerning the use of DNA lesion specific repair enzymes, only EndoIII reflected the ability of glyphosate (considering its higher concentration) in exert oxidative damage (oxidized pyrimidines), suggesting that oxidation of DNA bases was not a dominant mechanism of damage. Complementary, the overall oxidative damage ($GDI+NSS_{FPG}+NSS_{EndoIII}$ parameter) confirmed the genotoxicity of glyphosate and AMPA, considering all the tested concentrations and exposure times. Overall, the present findings pointed out the genotoxic hazard of tested chemicals to fish, with AMPA displaying a genotoxic potential comparable to its precursor (glyphosate). These findings reinforce the importance to consider the degradation products in risk assessment plans concerning pesticides in the water systems.

TU065

Identity and synthesis of the major egg yolk proteins of the fresh water mussel Unio tumidus and its occurence and inducability during a full reproductive cycle.

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The major egg yolk proteins of *Unio tumidus* has been purified and characterized by mass spectrometry. An ELISA for measuring the yolk proteins has been developed and compared to measurements by the Alkali Labile Phosphate method. The site of synthesis of the yolk protein has been investigated using *in-situ* hybridization. The developed tools, together with histology, have been employed for describing the occurence of the yolk proteins in males and females during a full reproductive cycle. Further, the inducability of the yolk proteins by estradiol has been investigated in exposure experiments.

TU066

Effects of chlordecone on endocrine system of Macrobrachium rosenbergii

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Effects of endocrine disruptors (e.g. industrial chemicals and pesticides) are well documented in vertebrates, but less studied in invertebrates, while these organisms represent the major part of the aquatic biota. Crustaceans are one of the most ubiquitous groups of aquatic invertebrates, and represent good models in ecotoxicological studies that evaluated the effects of endocrine disruptor compounds. Individual of the crustacean species occupy various ecological niches and their success, in part, stems from neuro-endocrine signaling cascades that regulate physiology in response to environmental and internal pressure. Ecdysteroid hormones, such as 20-hydroxyecdsyone, are the major signaling molecules which regulate various physiological processes such as reproduction and molting in crustacean. In addition, chitobiase is a chitinolytic enzyme involves in exoskeleton degradation in arthropods and thus plays an important role in molting and growth of crustaceans. Disruption of the ecdysteroid signaling pathways and chitobiase activity in crustaceans has been associated with aberrations in growth, metamorphosis, sexual development and sex determination that are indicative of environmental endocrine disruption. In this study, we investigated the effects of the chlordécone (Kepone), an organochlorinated pesticide, on the endocrine system of *Macrobrachium rosenbergii* by measuring chitobiase activity and 20-hydroxyecdsyone (20HE) concentrations in muscle. Post-larva of *Macrobrachium rosenbergii* were exposed to chlordecone at nominal concentrations (0.02, 0.2, 2, 20 µg L−1) for 768 hours. The results revealed a decrease of the 20HE concentration according to the time and the concentration of exposure. On the other side, the chitobiase activity was increased at the highest exposure concentration. The results suggest that endocrine disruption could occur in *Macrobrachium rosenbergii* following an exposure to an estrogenic compound such as chlordecone. The ecdysteroid concentration and the chitobiase activity disturbance allow us to better understand the mechanisms of action of endocrine disruptor compounds in crustaceans.

TU067

Acute and chronic effects of selected pharmaceuticals on the chlorophyll fluorescence intensity and photosynthetic yield of Raphidocelis subcapitata and Chlorella vulgaris

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The excess of absorbed light energy used in driving photosynthesis, re-emitted in the form of chlorophyll fluorescence is often used as an indicator of photosynthetic performance in photoautotrophs. In an effort to assess the acute and chronic toxicity of selected human pharmaceuticals on freshwater phytoplankton, *Raphidocelis subcapitata* and *Chlorella vulgaris* were exposed to varying concentrations of amoxicillin, cyclophosphamide, lidocaine, ifosfamide and sulfamethoxazole and their individual effects on the photosynthetic yield, variable fluorescence and the steady state fluorescence of photosystem II were investigated. Acute sulfamethoxazole exposure caused a significant decrease (p< 0.05) in the photosynthetic yield, variable fluorescence and steady state fluorescence of the phytoplankton. *C. vulgaris and R. subcapitata* acutely exposed to lidocaine showed a decline in photosynthetic yield and variable fluorescence. However, acute exposure of both organisms to cyclophosphamide, ifosfamide and amoxicillin did not alter significantly, the chlorophyll fluorescence yield and intensity except in *R. subcapitata* at the highest tested concentration of cyclophosphamide. Stimulatory effect or hormesis was provoked at some of the tested pharmaceutical concentrations. In the chronic studies, exposure of both organisms to sulfamethoxazole, lidocaine, and amoxicillin led to a significant reduction (p< 0.05) in the photosynthetic yield and variable fluorescence. Ifosfamide and cyclophosphamide had no effects on the fluorescence yield and intensity in *R. subcapitata* while they induced significant effects (p< 0.05) on the photosynthetic yield and steady state fluorescence in *C. vulgaris* respectively. Acute and chronic exposure to sulfamethoxazole and lidocaine elicited immediate adverse effects on the photosynthetic performance of the organisms with amoxicillin showing delayed effects. Although the concentrations used in this study do not reflect the expected concentrations in the environment, sulfamethoxazole was the only pharmaceutical that caused a marked reduction in all the photosynthetic-fluorescence parameters following chronic exposure to the lowest tested concentration.

Innovations in environmental analytical chemistry: the quest for pollutants at trace levels (P)

TU068

3iTOX: A novel approach for monitoring water toxicity

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3iTOX project aimed on the development of a fully automated Solid Phase Extraction (SPE) technique suitable for 356 compounds followed by toxicity assessment using TOXcontrol. TOXcontrol (microLAN, The Netherlands) is an online biomonitor for continuous toxicity assessment using the bioluminescent bacteria *V.fischeri*. The driving force of this study was the finding of very low concentration of contaminants in the surface water of the Netherlands, which resulted in no response to conventional bioassays [1]. An extensive research in identifying the most applicable SPE sorbent giving the highest recoveries after extraction of 10ml samples using Symbiosis™ coupled to Quadrupole Time of Flight Liquid Chromatography-Mass Spectrometry (QTOF LC-MS) revealed that Oasis® HLB material was the most suitable for such a wide range of compounds [2]. This method was scaled to the SPE unit of TOXcontrol, the newly developed sensor platform for pre-treating samples with SPE prior to toxicity assessment. Initially the method was validated using the SPE material of the manufacturer (Hocer, Nantes, France), which proved of comparable efficiency as Oasis® HLB, when eluting with 2,5ml methanol in total. Since the results using the SPE unit of TOXcontrol were close to those achieved while using advanced equipment in the laboratory, it was tested with real samples. In particular, this method was applied in drinking and surface water, blank samples and spiked with the same 356 organic compounds including pharmaceuticals, pesticides and industrial compounds, polar and non polar compounds. This method runs completely automatically and with applicability for online measurements at the point of interest.

TU069

Optimisation and validation of an on-line TFC/UHPLC-MS/MS method for the analysis of perfluoroalkyl acids in biota and sediment samples

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The cost-effectiveness of the analytical procedure is becoming crucial in all laboratories. Turbulent Flow Chromatography (TFC) is a technique that can be implemented as an online clean-up increasing productivity and reducing solvent consumption without sacrificing sensitivity. The present study presents the development and validation of on-line TFC extract clean-up procedure coupled with UHPLC-MS/MS multi-residue method for analysis of determination of 8 perfluoalkyl carboxylates (from 5 to 12 carbon atoms) and 3 perfluoalkyl sulfonates (from 4 to 8 carbon atoms) in biota and sediment samples. Fast sample preparation

procedure was based on a sonication-assisted extraction of sample with ACN/H_2O mixture enhanced by salting out and acidification. The extracts were on-line cleaned up by TFC and elution of the analytes was achieved in isocratic focusing mode. The main advantages of the method are the possibility of injecting 100% solvent extracts without having to reconstitute with the eluent aqueous phase, the increased method robustness and productivity because of reduced sample handling and enhanced sample clean-up. The stationary phase, mobile phase composition, flow rates and injected volume of the online TFC clean-up procedure were optimised in order to achieve the best sensitivity. The method was validated with clam samples. Quantification was achieved by solvent external calibration. Matrix effect was evaluated analysing fortified extracts: signal suppression ranged from 1 to 60%, thereby isotopic dilution was implemented to improve the accuracy. Validation was carried out by analysing spiked clam samples since no certified reference materials were available for PFAS in these matrices. Recovery values were between 98 and 133 % for the perfluorocarboxylates and between 40 and 60 % for the perfluorosulfonates. LODs and LOQs ranged from 0.03 to 0.3 ng/g ww and from 0.1 to 0.9 ng/g ww respectively. Repeatabilities (intra-day precision) and reproducibilities (inter-day precision) showed RSD from 3 e 13% (average value of 6%) and from 4 e 27% (average value of 13%) respectively.

TU070
Validation of a quantitative method for lipophilic marine toxins in shellfish proves power of UHPLC-HR-Orbitrap mass spectrometry.
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Lipophilic marine toxins produced by microalgae can be accumulated in edible filter feeders such as shellfish, leading to an introduction of toxins into the human food chain producing different poisoning effects. During the last years, many analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have been consolidated by inter-laboratory validations. However, the main drawback of LC-MS/MS methods remains the limited number of compounds that can be analysed in a single run. Moreover, due to the targeted nature of LC-MS/MS only known toxins for which methods have been previously optimized will be detected. Therefore in this study, a method based on ultra high-performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry was developed and its quantitative performance evaluated for confirmatory analysis of regulated lipophilic marine toxins in shellfish flesh according to Commission Decision 2002/657/EC and EU Reference Laboratory for Marine Biotoxins SOP 2011. Okadaic acid (OA), dinophysistoxin-1 (DTX-1), pectenotoxin-2 (PTX-2), azaspiracid-1 (AZA-1), yessotoxin (YTX) and 13-desmethyl spirolide C (SPX-1) were quantified using matrix-matched calibration curves. For all compounds the RSD_R ranged from 2.9 to 4.9%, repeatability from 2.9 to 4.8 % and recovery from 90% to 112% for three spiked levels. In addition, a first confirmatory identification of the compounds was performed by detecting the $[M+H]^+$ or $[M-H]^-$ ion with their specific retention times and accuracies. Secondly, the $^{13}C/^{12}C$ diagnostic isotopic ratio was selected for confirmation of a compound's identity. In conclusion, UHPLC-HR-Orbitrap MS permitted more accurate and faster (less than 7 min.) detection of the target toxins than previously described LC-MS/MS methods. Furthermore, HRMS allows to retrospectively screen for many toxin analogues and metabolites using its full scan capabilities but also untargeted screening through the use of metabolomics software.

TU071
A novel analytical method for determining the prevalence of anticoagulant rodenticides in non-target species
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Anticoagulant rodenticide use is widespread not only in agricultural settings but also in urban environments. Because of this diverse use, non-target species can be exposed to these chemicals in a variety of ways. Through residue analysis, numerous studies have shown that often non-target wildlife have exposure to multiple anticoagulant rodenticides whereby complicating risk assessments. Although more studies need to be done to determine the potential increases in toxicity when animals are exposed to combinations of rodenticides, it is also important to understand the scope of exposure to general populations of non-target species. Therefore, we developed a method to quantify twelve anticoagulant rodenticides in whole blood using high-performance liquid chromatography combined with electrospray ionization tandem mass spectrometry (HPLC-MS/MS). Nine hydroxycoumarin derivatives (coumafuryl, warfarin, coumatetralyl, coumachlor, bromadiolone, difenacoum, flocoumafen, brodifacoum, difethialone) and three 1,3-indandione derivatives (pindone, diphacinone, chlorophacinone) were analyzed over the concentration range of approximately 2-750 ng/mL whole blood. These anticoagulant rodenticides were selected based on registration status; all of them have been registered for use in the United States, Canada, India, Australia, New Zealand, and/or Western Europe. Data will be presented on the incidence of anticoagulant rodenticide residues in blood drawn from non-target birds using this method.

TU072
Active in situ sampling for bioavailability using solid phase extraction - the IS2B
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Contamination of surface water sediments with persistent chemicals like insecticides, pharmaceuticals, and antimocrobials is an ongoing concern because these pollutants can be toxic, endocrine-disrupting, carcinogenic, and bioaccumulative. It is therefore important to determine the bioavailability of these contaminants in their environmental sink, i.e., in aquatic sediments. Direct analysis for bioavailability of environmental contaminants can be cumbersome because it requires the exposure, capture, and subsequent analysis of biota living in contaminated environments to accurately determine body burdens associated with sediment pollution. In the present, ongoing study, we explore the risk posed by sediment-borne contaminants, including fipronil, triclosan, triclocarban, *p,p'*-DDE, and dieldrin. We have designed an active sampling device that includes a chemical surrogate for bioavailability studies in the form of a solid phase extraction (SPE) resin containing hydrophobic ligands. A stainless-steel tube containing a six-channel peristaltic pump is placed into contaminated sediment, and the pump separately delivers sufficient amounts of pore water and bulk water (mL to L) through an array of SPE cartridges, thereby concentrating dissolved chemicals from the aqueous phase that is assumed to be representative of the bioavailable fraction of the total mass of contaminants present. The SPE cartridges act as surrogates for biota in this innovative device. The contaminant mass captured with the SPE resins in situ can then be analyzed in the laboratory via GC-MS/MS or LC-MS/MS and compared to the body burden of biota exposed in mesocosm experiments. Direct comparisons of the active sampler performance with that of passive sampling sorptive stir bars (coated with polydimethyl siloxane) indicates that at a flow rate of 70 uL/min, the mass uptake rate of hydrophibic organics in NOM-laden water is 1.3 - 170 times faster in the active sampler. The intent of the project is to formulate and validate mathematical models that enable one to translate measurements obtained with the *in situ* sampler for bioavailailability (IS2B) directly into body burden estimates for relevant aquatic macrobiota, thereby informing environmental risk assessments without the need for sacrificing animals in the process.

TU073
Analysing highly hydrophilic micropollutants in water by direct injection LC-MS/MS
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Hydrophilic micropollutants are of particular concern with regard to drinking water contamination, as current water treatment processes often have low removal efficiencies. Their analysis at trace levels is also a challenging task, as a preconcentration by solid-phase extraction is difficult due to their low affinity towards commonly used polymeric sorbents. While ion exchange materials or activated carbon sorbents were used with some success, a breakthrough of compounds resulting in low recoveries due to matrix components is often observed. To avoid the limitations of solid-phase extraction we developed a direct-injection LC-ESI-MS/MS method for the analysis of highly hydrophilic, neutral and ionic contaminants. As a large-volume injection of water samples was used to increase sensitivity, reversed-phase LC was the technique of choice and a sufficient chromatographic retention could be obtained using a polar-embedded stationary phase. Using this method we were able to analyse a range of contaminants such as pesticides and metabolites (e.g., mepiquat, chlormequat, desphenyl chloridazon), drugs (e.g., fluorouracil, metformin) and industrial chemicals in water samples at levels below 10-100 ng/L.

TU074
Characterisation of Combined Sewer Overflow Discharge upon the River Thames using a Semi-Targeted Liquid Chromatography-High Resolution Mass Spectrometry Screening Method
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London is served by a Victorian sewage system that struggles to cope with the demands of an ever increasing population (now at 8.2 m), especially at times of high

rainfall. As a consequence, approximately 39 million tonnes of raw sewage are discharged directly into the Thames River every year via combined sewer overflows (CSOs). Such discharges bypass waste water treatment processes and hence, represent a potentially significant point source of environmental pollution including pharmaceutically related contaminants, which have been shown to be bioactive at low concentrations. A semi-targeted (target and non-target) wastewater screening approach for the detection and confirmation of a range of over-the-counter, prescribed and illicit drugs is presented herein using solid phase extraction (SPE) and liquid chromatography-high resolution mass spectrometry (LC-HRMS). A number of structurally diverse model species deriving from analgesics, antibiotics, antipsychotics, antidepressants and illicit drugs were used to develop a suitably broad targeted quantitative analytical screening method that was subsequently applied to the differential analysis of influent/effluent wastewater and river water. The use of HRMS (Orbitrap technology, resolution = 50,000 FWHM) permitted simultaneous qualitative semi-targeted analysis of all matrices for pharmaceutical/drug content. Relevant species were confirmed by their accurate monoisotopic m/z ratios (parent and fragment ions) using reference standards to confirm chromatographic retention time where possible. The developed method displayed excellent linearity (R^{2-} all >0.98), reproducibility (e.g. all < 20 % RSD in river water) and recovery (>75 %) for target analytes in matrix. Several targeted compounds were determined at ng/L levels in all environmental samples as well as a range of other pharmaceutical species, including both prescription and illicit drugs. The subsequent performance of the method for these compounds is also presented. The developed method was finally applied to the differential identification of compounds present in influent and river water to identify potential CSO event markers. This semi-targeted approach highlights the potential of HRMS as a novel detection technique in the identification of emerging contaminants.

TU075
Determination of xenobiotic residues in the fresh water invertebrate, Gammarus pulex, using agitated solvent extraction, solid phase extraction and liquid chromatography tandem mass spectrometry
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Xenobiotics and their metabolites are continually discharged into aquatic environmental compartments mainly via sewage treatment plant effluents. Many of these compounds are bioactive in nature and can result in acute and chronic effects on reproduction, development and behaviour in biota.[1] Their toxicity varies considerably due to the combination and diversity of compounds present and much more knowledge is required to understand their potential (or not) for bioconcentration in selected species.[2] However until now, the multi-residue determination of xenobiotics in small biota at the ng/g level has been analytically challenging. Herein, we present a new analytical method for the confirmatory identification and quantification of 16 xenobiotics in the aquatic invertebrate, *Gammarus pulex*. Collected Gammarids were weighed, washed, freeze-dried at -50 ^oC and homogenised before extraction in 5 mL acetonitrile in a ball mill micro-extraction vessel. Extracts were then purified and concentrated by solid phase extraction using a Waters Oasis HLB sorbent (200 mg, 6 mL barrel). Extracts were analysed using an optimised reversed-phase liquid chromatography-tandem mass spectrometry method in positive and negative electrospray ionisation modes. Method performance data is also presented herein showing acceptable linearity ($R^2 \geq 0.98$; range from 10 ng/g to 10 µg/g dry weight in most cases), reproducibility (RSD ≤ 20 %) and limits of quantification (3-57 ng/g). This method was successfully applied to the trace determination of several xenobiotic residues present in Gammarids across multiple river locations in the South London area at ng/g concentrations showing that the method was fit for purpose. Occurrence of xenobiotic residues in surface water from each site is also shown. This study shows that xenobiotic contamination was present in *Gammarus pulex*. This validated analytical method will enable measurement of xenobiotics in future exposure studies. **References.** 1)Daughton, C.G. & Ternes, T.A. (1999). *Environ. Health. Persp.*, 107(6), 907-938. 2) Madureira, T. V., et al. (2011). *Aquat. Toxicol.* 105(3-4): 292-299.

TU076
Determination of Paraben on Mogi River by dispersive liquid-liquid microextraction (DLLME) by HPLC with diodo array detector
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Parabens are alkyl hydroxyl benzoate parabens preservatives. They are extensively used to prevent microbial growth in many common care products, such as cosmetics, underarm deodorants, hair products, pharmaceuticals, and some foods. Most parabens were frequently found in river water at concentrations reaching from ng L^{-1} to mg L^{-1}, and their levels depended mainly on the extent of water dilution resulting from rainfall. Discharge of treated wastewater effluent into the river course was found to be the main cause of water contamination with parabens. Dispersive liquid-liquid microextraction (DLLME) is an analytical technique, based in the dispersion of extraction solvent assisted with a disperser solvent within an aqueous solution that generates a very high contact area between the aqueous phase and the extraction solvent. The present study reports the development and application of a DLLME-HPLC-DAD method for the simultaneous separation and determination of four paraben preservatives (methyl-, ethyl-, propyl-, and n-butyl-paraben) in surface water. The surface water samples were collected in spring season, from four cities in Low and Medium Mogi River region (Guatapará, Rincão, Porto Ferreira, and Pirassununga), and two cities from High Mogi River region (Mogi-Guaçu, and Itapira), in São Paulo state, Brazil. The DLLME-HPLC with diodo array detection method shows a good chromatographic separation for the 4 parabens studied (methyl-, ethyl-, propyl-, and n-butyl). The method exhibit detection limits from 1.0µg L^{-1} for methyparaben, 1.0µg L^{-1} for methylparaben, 0.8µg L^{-1} prophylparaben, and 1,0µg L^{-1} buthylparaben. Good recovery index for the extraction procedure, from 30.3% (methylparaben) to 68.0% (prophylparaben), and good reproducibility (> 95% RSD). Surface water samples collected from Medium and Fish Mogi River region present a median content of parabens of 3.53µg L^{-1} (MP), 2.70µg L^{-1} (EP),and 15.2µg L^{-1} (PP), while those samples originated from High Mogi River region shows median levels of 21,8µg L^{-1} (MP), 13.3µg L^{-1} (EP), 14.5µg L^{-1} (PP), and 6.2µg L^{-1} (BP). **Keywords:** Paraben, water, dispersive liquid-liquid microextraction, and HPLC-DAD. **Acknoledgment:** This work was supported by CNPq, CAPES, and FAPESP.

TU077
Implementation of a complementary monitoring strategy to enable an efficient survey of pharmaceuticals in urban water cycle. A case study on the parisian area
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Investigative monitoring through the last decade has highlighted a ubiquitous contamination of water, all through its cycle, by pharmaceuticals residues. Nevertheless, the picture is incomplete or misses of reliability thus impeding proper risk assessments as well as efficient environmental and sanitary protection/prevention. Recently, the scope of attention has been widening to psychotropic drugs as well as illicit substances. Still, since risks on non-targeted biota are not clear yet, there is a great challenge in studying those compounds, which implies a twofold goal. The first is to keep providing occurrence data in aquatic environment for these molecules. The second is to scrutinise for novel ones and especially for metabolites and transformation products on which only scarce information is available despite their relevance. A monitoring strategy based on the simultaneous implementation of two complementary approaches enabling the monitoring 70 French' relevant psychotropic compounds -including 29 metabolites and glucuronides- in the water cycle is presented. On the one hand, a multi-residu SPE-UPLC-MS/MS method was successfully developed, enabling a reliable quantitation, at ultra trace level, of a broad range of benzodiazepines, hypnotic drugs, antidepressants, stimulants, opiates and opioids, anticonvulsants, anti-dementia drugs. Moreover, analgesic and non steroidian anti-inflammatory drugs were included as anthropic tracers. On the other hand, homemade polar organic compound integrative samplers (POCIS) were calibrated toward each analyte of the hereinabove reported method, thus providing a complementary sampling tool enabling to concentrate *in situ* trace pollutants and yielding in time-weighted concentrations. Both strategies were successfully implemented in the Paris area (Seine and Marne rivers watershed). Main results, advantages and limits of the implemented strategy as well as lessons will be presented and discussed.

TU078
Identification of pesticide metabolites on honey bees
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If it is assumed that pesticides play an important role in the decline of bees, it is still difficult to know the role of metabolites. This is mainly due to the lack of appropriate analytical method. The first difficulty is related to the extremely low levels of these by-products in bees, below the limits of quantification of current analytical methods. The second challenge is the lack of analytical standards, which are essential to develop quantitative methods and determine the environmental concentrations. Moreover, the structure of these products is not always known. The aim of this work is to identify the metabolites of pesticides belonging to the neonicotinoids, pyrethroids and carboxamids families, in bees. Pyrethroids and neonicotinoids have been recently introduced on the market, act at low doses per hectare and are undergoing increasing use. Carboxamids are fungicides largely used worldwide. In order to achieve identifying the metabolites, the proposed analytical strategy is as follows: the targeted pesticides are put in touch with the microsomes,

cell organelles responsible for the metabolism, to generate metabolites. These products of degradation are then characterized by complementary structural analyzes techniques (HR- MS , NMR ...) . This in vitro approach is complemented by in vivo studies in order to determine the kinetic aspects of bee's metabolism.
Keywords: pesticides, identification, metabolism, honey bees

TU079

Micro Flow UHPLC-MS/MS in Pesticide Analysis of Infant Foods

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Food safety with regards to infant food is of the upmost importance; however, it is also recognised as a challenging matrix to analyse due to the low maximum residue limit (MRL) of 0.01 mg/kg required by European Directive 2006/141/EC for all pesticides. Furthermore, the European Directive prohibits the use of certain very toxic pesticides in the production of infant foods and establishes even lower MRLs for a few other very toxic pesticides. Additionally, the analysis of infant food is complicated by their wide range of fat content. LC-MS/MS has been widely used for the quantitation of pesticides in infant food. The analytical methods typically use conventional LC flow rates (approximately 0.5 mL/min). Micro flow LC uses significantly lower flow rates (10 to 100 µL/min). With the same sample amount and identical LC peak width, the reduction in LC flow rate can result in an improved detection limit for concentration-dependent detection techniques such as electrospray ionization (ESI) mass spectrometry. Here, we utilise the improved response from micro flow LC to achieve the required low limits of detection for over one hundred pesticides in infant food. Initial validation results are presented for the micro flow LC method, in addition to robustness data. The developed micro flow LC methodology achieved the required MRL of 0.01 mg/kg for all 130 pesticides with all compounds eluted within 12.7 minutes. Initial validation data displayed excellent linearity for all compounds, low intra- and inter-day precision, no observed carryover, and good peak area and retention time stability over 48 hours. Micro flow analysis was successfully carried out a UHPLC system capable of both conventional higher flow rates and lower micro flow rates. Micro flow LC is a possible alternative to conventional flow LC if extra sensitivity is needed or reduction in solvent consumption is required.

TU080

Monitoring the presence of phosphodiesterase type V inhibitors in sewage water

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The presence of emergent contaminants in influent waters is no longer restricted to the environmental assessment of potentially toxic substances, personal care products, pharmaceuticals and illicit drugs. Recent publications are slowly crossing into the field of forensic evaluation of designer illegal analogs of lucrative voluntary-use drugs like the phosphodiesterase type V (PDE5) inhibitors Viagra®, Levitra® and Cialis®. The occurrence of all three erectile dysfunction treatment drugs has been reported in sewage water at very low concentrations (5-30 ng/L) mainly as the parent compounds. However, most of the literature on sildenafil pharmacokinetics in humans shows that after single oral or intravenous doses 90% of the drug was excreted as metabolites with no detectable parent in either feaces or urine. Consequently, the identification of metabolic transformations and analogues is particularly important to assess environmental distributions. The present work presents an analytical method developed for the detection of sildenafil with two metabolites and one analogue, tadalafil and three analogues, and vardenafil. The method employs solid phase extraction on OASIS® HLB SPE cartridges, followed by LC coupled to a high resolution Orbitrap mass spectrometer for the quantitative analysis of the analytes. Identification and quantification of target compounds was performed using the accurate mass spectra of the protonated molecule at a mass resolution of 30000 FWHM (m/z 400). For confirmation of the identity of the compounds at least one nominal mass product ion was used together with retention time, which was compared with that of the reference standards within 2.5%. The performance of the method was validated based on linearity of the response, recoveries, and method detection limits. The results of the application of the method to several wastewater samples are presented.

TU081

Effluent and surface water analysis of classical and novel drugs used in cancer treatment: 5-FU and protein kinase inhibitors

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Risk assessment of pharmaceutical products in the environment is currently covered by high uncertainties. On the one hand this is due to the lack of data, in particular on long term exposure and toxicity, on the other hand this may be attributed to the lack of consideration of additional parameters such as the exposure to mixtures and the presence of metabolite and/or transformation products (TPs). Especially regarding the occurrence of drugs used in cancer therapy, very limited data are available, as these compounds are not part of regular monitoring programmes. Presumably, the sometimes rather extreme physicochemical properties of a number of these compounds make it difficult to quantitatively determine them at sufficiently low levels in environmental matrices. To add to the knowledge on the occurrence of some pharmaceuticals that are used in the treatment of cancer, we have developed and validated a method for the quantitative analysis of 5-fluorouracil (5-FU), which can be regarded as a classical anticancer drug that has been in use for decades, usually administered to patients in a clinical setting. In addition, we have focused on the more recently introduced protein kinase inhibitors, such as imatinib, sorafenib, erlotinib and sunitib. These drugs are used more or less chronically as oral medication, without the need for in-hospital administration. In this poster, concentrations of 5-FU, imatinib, sorafenib, erlotinib and sunitib will be presented in effluents and surface water at various locations in The Netherlands.

TU082

Online SPE-nano-LC-HRMS of polar organic contaminants in environmental samples

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In nano-liquid chromatography (nano-LC), separation is performed at sub-microliter-per-minute flows over packed fused silica capillary columns, and coupling to electrospray ionization (ESI) can be achieved by direct spray without auxiliary gases. This technology promises high sensitivity through improved transmission and reduced matrix effects, and is becoming commonly used in proteomics applications. However, it is currently only rarely applied in environmental analytics. We present an analytical multi-residue method for polar organic contaminants in environmental and biological matrices. The method covers typical pesticide and pharmaceutical contaminants as well as their degradation products with a log Kow range between 2 and 6. In our approach, online solid phase extraction (SPE) of small sample volumes using a custom-packed cartridge is combined with nano-LC coupled to ESI-high resolution mass spectrometry. The cartridge is loaded with 20-100 uL sample in aqueous matrix, subsequently, the cartridge is eluted with methanol and the eluate flow is mixed with water for refocusing on the analytical column. The analytical column is then eluted with a water/methanol gradient with a flow of 700 nL/min and coupled to an Orbitrap (Thermo-Fisher) Fourier-Transform mass spectrometer for detection. The method shows high sensitivity, enables the use of small sample volumes and reduces solvent consumption. This is one of the first applications of nano-LC for a broad range of compounds in environmental analytics.

TU083

Pharmaceuticals and PCPs in groundwater: Results from French National screening

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In 2011, the French Ministry of Ecology and the Water and Aquatic Environment National Office have implemented a national screening campaign in groundwater focused on occurrence of "emerging" organic contaminants with various potential uses and origins. Among the 411 Organic compounds (OC) targeted, 131 pharmaceutical products, 13 life style products and 10 cosmetics have been selected and measured in in 2011 during two sampling campaigns (at high and low water levels), representing 960 measurements per compound. Thirteen pharmaceuticals have been detected with quantification frequencies up to 1%. Amongst them, some are expected such as carbamazepine and acetaminophen while others are less expected such as metformin and tramadol. Concerning personal care products and life style products, caffeine is the most frequently OC detected (40%) with cotinine (6%). Although the concentrations of octocrylene, propylparaben and galaxolide can reach 60 ng/L, 103 ng/L and 120 ng/L respectively in some sites, their occurrences, at the national-scale, are lower (below 1%). By contrast, other musks are rarely detected but their concentration may exceed 400 ng/L, with a maximal concentration level of 1.3 µg/L for musk ketone. Quantification frequencies obtained in this study are comparable with those of previous studies [1-3] with similar choices concerning sampling strategy and targeted molecules. The impact of these criteria and of reporting levels on quantification frequencies will be discussed. 1. Loos, R., et al., *Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water*. Water Research, 2010. **44**(14): p. 4115-4126. 2. Teijon, G., et al., *Occurrence of emerging contaminants, priority substances (2008/105/CE) and heavy metals in treated wastewater and groundwater at Depurbaix facility (Barcelona, Spain)*. Science of the Total Environment, 2010. **408**(17): p. 3584-3595. 3. Standley, L.J., et al., *Wastewater-contaminated groundwater as a source of endogenous hormones and pharmaceuticals to surface water ecosystems*. Environ Toxicol Chem, 2008. **27**(12): p. 2457-68.

TU084

The LC-ESI-MS/MS method of simultaneous detection of 21 antidepressants in surface water samples and its application in assessing the presence of the pharmaceuticals in two rivers of central Poland

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Antidepressants even at low concentrations can reveal some adverse effects on aquatic life due to disturbing homeostasis throughout the central and peripheral nervous system both in vertebrates and invertebrates. So far there have not been any reports regarding the presence of these pharmaceuticals in surface waters of Eastern Europe. Therefore we aimed to create the method based on liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) of simultaneous detection of 21 antidepressants in surface water samples to assess the presence of the active compounds at specific points before and at the effluent discharge of the main Polish river – the Vistula, a smaller river of the Warsaw region – the Utrata. Samples were collected twice at one month intervals. The target compounds were extracted using solid-phase extraction on HLB OASIS (Waters). The LC-MS/MS was operated under the multiple reaction monitoring (MRM) mode. The method developed was both sensitive and accurate. The method limit quantitation was between 0.5 (for clomipramine) and 6.1 ng/l (for sertraline). The highest concentration of antidepressants was observed in the small river Utrata. The antidepressants found in highest concentrations in both rivers were venlafaxine (up to 250 ng/l), moclobemid (up to 45 ng/l) and citalopram (up to 17 ng/l). This project was funded by National Science Centre, NCN (2011/03/B/NZ7/00751).

TU085

EU Water Framework Directive demands on emerging contaminants water analysis – suspended organic matter and particulates effects on bulk samples

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Rather than "newly introduced substances", the term "contaminants of emerging concern" (CEC) is used to describe contaminants environmentally persistent and with potentially harmful human or ecological effects, or because a new source or a new pathway to humans was discovered. These CECs have no published health standards and are not usually included in monitoring programs because their presence and significance assessment is very recent [1]. Musks and alkylphenols are two families among the CECs. Musks are known for their ubiquitous presence in the environment as a result of their extensive use as fragrance ingredients in personal care products. On the other hand, alkylphenols are extensively used in as precursors to the detergents, as additives for fuels, lubricants, polymers and rubber, making their constant presence a rising concern. Currently, the EU Water Framework Directive (WFD) demands that total concentration of organic pollutants should be considered in water analysis, although only dissolved metal concentration should be monitored and evaluated in order to satisfy the Environmental Quality Standard (EQS). It is known that substances with a K_{ow} above 3 are considered hydrophobic and therefore are more likely to be settled in the suspended organic matter, sediments or biota [2]. To face this challenging WFD demand, water analysis to determine musks and alkylphenols was performed in both estuary and wastewater samples where dissolved and bulk matrices were compared, using deuterated surrogates as internal standards, and the interactions between these compounds and suspended organic matter and particulates was evaluated. **References** [1] USEPA. http://water.epa.gov/scitech/cec/ Accessed: 29 November 2013. [2] Coquery et al. 2005. Priority substances of the European Water Framework Directive: analytical challenges in monitoring water quality. Trends 24:117-127.

TU086

Application of Information Dependent Acquisition experiments in LC MS/MS method to identification of biotransformation products of selected anti-depressants

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Psychiatric pharmaceuticals such as antidepressants are among the most prescribed active substances throughout the world. Many pharmaceuticals undergo structural changes in human bodies e.g. enhancing water solubility. In spite of some metabolites have a high biological activity, they have not been determined in environmental samples, mainly due to the lack of analytical standards. The main goal of the project was the development of a method for identification of antidepressants' biotransformation products in environmental samples. In the first step of the research, the selected pharmaceuticals were incubated with rat liver s9 fraction. The parent drugs and the metabolites were extracted and purified. Drugs concentrations were monitored by liquid chromatography hybrid triple-quadrupole linear ion trap mass spectrometers (4000 Q TRAP AB Sciex) in the positive and negative electrospray ionization (ESI+ and ESI-) with two MRM transitions (a quantifier and a qualifier ion for each compound). Detection of the biotransformation products was performed using Information Dependent Acquisition (IDA) experiment. An IDA method automatically runs experiments based on results obtained from previous experiments. IDA contained two Precursor Ion scan and specific IDA criteria which were optimized for each compounds. After IDA criteria was added experiments Enhanced Product Ion and Enhanced Resolution. On the basis of IDA results, MRM transitions of biotransformation products were designated. Drug concentration and the level of the metabolites were monitored using MRM transitions and IDA experiments. The developed method was applied for the analysis of antidepressants and their metabolites in Vistula river near Warsaw. Project was founded by National Science Centre, Poland, grant no DEC-2011/03/B/NZ7/00751.

TU087

Detection and identification of ozonation products in water by non-target approach

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The efficiency of ozonation for the removal of many micropollutants from water is already proven. However, there are still gaps in knowledge on their transformation products, as well as in finding new and emerging pollutants and their fate during ozonation. The development of analytical tools like HR-MS/MS and the application of differential analysis has made the non-target approach more effective. The aim of this work was the detection and identification of ozonation products (OPs) from two pharmaceuticals and the anticorrosive benzotriazole which are abundant in the environment in batch experiments. The experimental solution contained 0.04 mM of investigated compound. Hydroxyl radicals were quenched using 20 mM t-BuOH in water. The solution was maintained at pH 7 with a 50 mM phosphate buffer. The experimental mixtures were ozonated under different compound-to-ozone ratios, ranging from an excess of compound (2:1 molar) to an excess of ozone (1:10 molar). The experiments were performed until the depletion of ozone was complete. Subsequently the OPs were analyzed by LC-HR MS/MS. Separation of OPs was achieved with an Atlantis® T3 3μm 3.0 x 150 mm column. Analytes were eluted with a gradient program using MeOH and water, both acidified with 0.1% formic acid. MS data were acquired with a ThermoScientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer. MS data were collected parallel in full scan mode (60-700 m/z) at 70,000 resolution and in data-dependent MS^2 mode at 17,500 resolution, using both positive and negative electrospray ionization. OPs were detected by differential analysis using the SIEVE Software (ThermoScientific™). Non-spiked/ozonated and spiked/non-ozonated solutions of the compounds served as the control samples. So far, the results from the OPs of benzotriazole reveal the presence of five products. Among OPs which have been already described in the literature, also two new OPs - 1*H*-1,2,3-triazole-5-carbonyl-4-carboxylic acid and 1*H*-1,2,3-triazole-4,5-dicarboxylic acid- were found. Proposed approach is efficient for detection and identification of ozonation products and will be used in further investigation.

TU088

Elucidating exposure pathways of phthalate esters in the indoor environment: combining measurement and modelling techniques.

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Phthalate esters (PEs) are widely used as additives to obtain suitable flexibility and durability of polymeric products. Since they are not covalently bonded to the material they can easily get released into the air or leach from the products, contaminating the environment. The present work is part of the 'Advanced Tools for Exposure Assessment and Biomonitoring' (A-TEAM) project within a Marie Curie Initial Training Network. The project aims to develop methods for measuring external and internal human exposure of four substance groups, among those the phthalate esters. The study group comprises 60 adults from households in the Oslo area. The experimental work includes determination of phthalate esters in indoor air, house dust, dietary items and handwipes to investigate the contribution of different external human exposure pathways such as inhalation, dietary and dust ingestion and dermal absorption in the Norwegian cohort. Phthalate metabolites in human urine and saliva will be analysed using liquid chromatography/mass

spectrometry (LC/MS/MS) in order to determine the internal dose. In combination, exposure modelling techniques will be applied to elucidate the relative importance of indoor air, dust ingestion, dermal uptake and dietary intake as exposure pathways for phthalates. As a part of this study, active stationary and personal indoor air sampling for 24 h was performed to obtain representative air samples. Solid-phase extraction (SPE) cartridges gave a fast and easy method, eliminating time-consuming sample preparation steps (Bergh et al. 2010; Staaf and Ostman 2005). House dust samples were collected from settled dust on elevated surfaces, as well as floor dust and dust from vacuum cleaner bags. Analysis of the dust included a sieving step followed by an ultrasonic-assisted solvent extraction for the analytes of interest (Abb et al. 2009; Bergh et al. 2012). A novel method utilising hand wipe samples made from glass wool was used to assess the total amount of phthalates on both hands of the participants. A selective and sensitive method to determine phthalate esters was obtained by using gas chromatography/triple quadrupole mass spectrometry (GC/MS/MS) in multiple reaction monitoring (MRM) mode. The phthalate blank problem resulting from lab- and cross-contamination was minimized through application of a very short and fast sample preparation. Results from these initial measurements will be presented.

TU089

Analytical Method to Determine Precursors to Poly/Perfluorinated Compounds in Air and Water by GC-MS

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Different precursors to poly/perfluorinated substances can be present in the environment. These compounds can be the unreacted species present in the final products that are leached into the air and water. In this study, a gas chromatographic method with mass spectrometric detection was developed and validated to determine representative precursor compounds that include: perfluorinated alkyl iodides (PFAI), fluorotelomer iodides (FTI), fluorotelomer olefins (FTO), fluorotelomer acrylates (FTAC) and fluorotelomer methacrylates (FTMAC). The positive ions produced during electron impact ionization were measured. Method validation results showed high linearity and sensitivity in the selected ion monitoring (SIM) mode. The absolute instrumental detection limits were in the range of 0.5 to 2 pg. A solid phase extraction technique was optimized using available stionary phases for enrichment. A sampling plan was developed so that the method detection limits are 1 pg/L in air and 10 ng/L in water.

TU090

Analysis of brominated, chlorinated and phosphorous flame retardants using GC-MS, GC-MS/MS and GC-TOF-MS

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Flame retardants (FRs) are chemical compounds widely used in many different materials in order to provide fire protection. FRs are for instance used in textiles and plastics and are added to everyday-life products such as furniture, computers and building insulation. Due to their persistent, bioaccumulative and toxic properties, many FRs are substances of concern in the environment. The polybrominated diphenyl ethers (PBDEs), previously used in high volumes, have been detected in the Arctic far away from emission sources which proves their high persistence. The use of PBDEs in new materials has been banned by the European Union (EU) and will be phased out by the main producers in the US until the end of 2013. This has led to the production of new FRs, some of which are believed to have similar properties as the PBDEs. Therefore, there is a need to develop new analytical methods in order to analyze brominated (BFRs), chlorinated (CFRs) and phosphorous FRs (PFRs) in the environment. This study focuses on the analysis of approximately 50 FRs using gas chromatography (GC) coupled to single mass spectrometry (MS) with chemical ionization (CI), quadrupole time-of-flight MS (TOF-MS) with electron ionization (EI) and tandem-MS with both EI and CI. The different MS techniques were tested on sediment samples and compared in terms of sensitivity, selectivity, repeatability, linearity and identification capability for the analysis of FRs.

TU091

Development of a one-step integrated pressurized liquid extraction and cleanup method for determining polycyclic aromatic hydrocarbons in marine sediments

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A rapid and accurate one-step integrated pressurized liquid extraction (PLE) and cleanup method was developed and validated for 34 polycyclic aromatic hydrocarbons (PAHs) in marine sediments, giving an extract that could be analyzed by gas chromatography-mass spectrometry without further cleanup. Marine sediment (5 g) was loaded into the stainless-steel extraction cell above activated copper (5 g) and activated silica gel (5 g). An extraction temperature of 100 °C and two 5 min extraction cycles using a 4:1 (v/v) hexane-dichloromethane mixture gave a good extraction efficiency. The integrated method gave extracts that were as clean as those obtained using PLE, followed by separate activated copper and silica gel cleanups. The method was validated, in terms of its accuracy and precision, using a certified reference material (NIST SRM 1944) and marine sediments spiked at low and high concentrations. The mean recoveries were 92% and 94% for the low and high spike concentrations, respectively, and the accuracy was good (giving a mean of 86% of the certified reference material concentrations). The method developed gave a precision and accuracy equal to or better than the precision and accuracy found using PLE with separate cleanups. The method developed gives a shorter sample preparation time and uses much less solvent than PLE and separate cleanups

TU092

Quantification of azole and pyrethroid pesticides in extracts of D. magna using QuEChERS and GC-ECD – method development and matrix effects

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Pyrethroid insecticides are highly toxic to aquatic invertebrates and exhibit EC50-values (acute, 48h) down to the low ng/L range. Recent studies showed that the high toxicity of pyrethroids can be increased by azole fungicides at environmentally relevant conditions. In order to understand the toxic action of these pesticide mixtures, highly sensitive and stable analytical methods, which are suitable for the quantification of trace levels of pesticides in small aquatic organisms, are needed. We present the development of a method for extraction, clean-up, and quantification of pyrethroid and azole pesticides in *Daphnia magna* using the "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) method in combination with GC-ECD. Matrix effects were tested and the method was validated for four azole and four pyrethroid pesticides. Highly challenging were the needs for very low detection limits (< 0.5 pmol/organism for pyrethroids) and the complex sample matrix causing a strong variation in the analyte response. In a first step, extracts from *Daphnia magna* were prepared with the QuEChERS method and spiked with analytes. Two dispersive SPE sorbent mixtures were compared: C18 and Primary Secondary Amine (PSA) and C18, PSA, and Graphitized Carbon Black (GCB). Samples were injected 30 times in a row and the course of analyte response was monitored. Analyte standards without daphnid matrix measured in between the matrix samples showed a strong increase or decrease in analyte response with the number of previous matrix injections (up to 43 % difference). On the contrary, the presence of daphnid matrix in the samples led to a stabilization of the peak intensities. The direction of the change in peak intensity was independent of the compound class and samples prepared with the two different sorbents exhibited similar behavior. Correlation tests were performed in order to identify analyte pairs exhibiting similar matrix effects. An improvement in response stability could be achieved if peak areas were normalized to correlating analytes. In a second step, accuracy, precission, and LOD/LOQ were determined. Recoveries with C18/PSA as sorbent were close to 100 % for all analytes, whereas recoveries for C18/PSA/GCB were rather low (30 – 80 %). The developed method (SPE sorbent: C18/PSA) was successfully applied to the measurement of trace levels of azole and pyrethroid pesticides in *D. magna* during *in vivo* uptake and elimination experiments.

TU093

Quantifying sampling artifacts for semi-volatile organic contaminants (SVOCs) in three active air sampling configurations and assessing inter-sampler comparability

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While many advances have been made in the analysis of trace environmental chemicals, advances in the air sampling of semi-volatile organic contaminants (SVOCs) have been more limited. Current active air sampler configurations are in fact similar to those used for the past 30 years. In addition, the SVOC community has not reached consensus on standardization of air sampling techniques within both global air monitoring programs and case studies. Data from different sampler configurations are often compared, and this requires knowledge of sampling artifacts such as breakthrough, filter blow-on/blow-off, and degradation. However, limited attention has been given to clarify this. The goal of this study was therefore to compare different active sampler configurations and evaluate the influence of sampling artifacts for a range of SVOCs. Three types of active samplers (high volume, low volume, and cascade impactor) were deployed together for periods of

1 to 7 days, concurrently with a continuous daily high volume sampler. Particles were sampled on QFF and the gas phase on 5 separate PUF slices to show the distribution of the compounds within sampler. All samplers deployed for >24 hours experienced breakthrough for more volatile compounds (especially low molecular weight polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and organochlorine pesticides). This was evidence through lower concentrations measured in samplers deployed for longer time periods or with higher flow rates, and a vertical distribution of compounds throughout all PUF layers. For example, samplers deployed for >5 days gave 30% lower concentration of PCB-28 than the average concentration from the daily high volume samplers deployed in the same period, while higher molecular weight PCBs were comparable to those measured in daily samples. Both experimental results and estimates suggest that at 25°C, high volume samplers may significantly underreport concentrations for PCB-28, α-HCH, and fluorene, for >600 m^3 air samples. Degradation was found to be a significant loss mechanism for PAHs and certain emerging contaminants, such as current use pesticides, particularly when sampling times exceed 24 hours and in areas with high ozone. All this has important implications for the protocols for global monitoring campaigns and particularly should be considered when data from different sampler configurations is being compared.

TU094

Fast GCMS Analysis of 60 VOC compounds using Headspace-Trap sampling

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The analysis of regulated volatile organic compounds in drinking and waste water is usually done with headspace or purge and trap technique using a so called 624 stationary phase with 30 m, 0.25 mm, 1.4 mm according to the EPA method 624. Reducing analysis time (fast GC) but maintaining chromatographic resolution has been successfully applied using narrow bore columns in various fields. However, the results reported were mainly based on liquid injection techniques. In Headspace analysis the transfer of sample from the insert to the column is usually relatively slow as mainly influenced by the gas flow through the insert. Regarding VOC analysis normally small split ratios are used in favour of sensitivity. This leads to a broad initial spatial band of the compounds over the first part of the column and therefore lead to bad chromatographic resolution which is in contradiction to fast GC approaches using narrow bore columns. For the present VOC analysis a film thickness of 1 mm was selected. The minimum inner diameter for such a column with a 624 phase available is 0.18 mm with a lengths of 20 m. The water samples (5 ml) were placed into 20 ml headspace vials and the volume injected was 1ml (split ratio 5:1). A cold trap is mounted at the top of the column directly under the injector in order to cool the first part of the column resulting in refocussing volatile compounds. The trap was cooled by direct liquid nitrogen and surrounds the column so that refocusing takes place at the top of the column. Different cold trap temperatures were selected and the optimum in refocusing was observed at – 150°C by measuring the peak profile at the end of the column in the mass spectrometric detector. As carrier gas Helium and Hydrogen was used. The best separation was achieved with Hydrogen at an average constant linear carrier gas velocity of 90 cm/sec. All 60 VOC peaks are separated in about 8 minutes run time. The peak width regarding one of the most volatiles which is venyl chloride was improved using the trap by more than a factor 50. The Limits of detection in real world samples for benzene and vinyl chloride were 0.01 and 0.002 mg/l, respectively. Using Hydrogen the sensitivity was reduced relative to Helium by a factor of 2 for the first eluting compound up to 5 minutes retention time and about 4 for compounds eluting later.

TU095

Determination of geosmin and 2-methylisoborneol in environmental water samples by headspace solvent microextraction and gas chromatography-mass spectrometry

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The identification and quantification of trace mounts of volatile organic compounds that cause taste and odour is essential since it was detected in environmental water, drinking waters,indoor air, fish tissues and foods. Geosmin and 2-methylisoborneol (2-MIB) are typically responsible for earthy-musty taste in all this cases. The odor threshold of these compounds for humans is 4 ng L^{-1} and 9 ng L^{-1} for geosmin and 2-MIB respectively. Hence, the sample preparation step is needed and a simple, rapid, clean and efficient extraction techniques are required.Head space micro extraction (HSME) based on the partition of analytes between an extracting organic solvent and the headspace above the aqueous sample solution. Standard solution of geosmin and 2-MIB were prepared in methanol (Sigma). A homemade HSME device were developedfor the preconcentration of geosmin and 2-MIB from aqueous samples. 5ml from the standard solution were poured into a 8ml vial, 0,3g of NaCl and 20μl off 1-octanol (organic solvent) wereadded. The sample vial were warmed up in constant temperature (40ºC) through by the sand inside the can which was in contact with the heated shaker for 10 minutes. After reaching the equilibrium, the microdrop was retracted into the syringe and injected into the GC-MS. The retention time of geosmin and 2-MIB were excellent if compared to other studies in the literature[2,3].

TU096

Pixel-by-pixel retention time shift correction of GC × GC-HRTOFMS data

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Comprehensive two-dimensional gas chromatography (GC × GC) enables separation of thousands of analytes in complex mixtures in environmental, biological and food samples. However, retention times (RTs) of the compounds in GC × GC often vary from run to run, due to carrier gas flow and/or temperature fluctuations, column condition, and matrices in the sample. The RT shifts affect automated data processing for comparison of chromatograms, as well as peak identification and quantification. To address this issue, several studies have developed algorithms to correct RT shifts in chromatograms generated by GC × GC coupled to uni-variant detectors. In this context, Gros et al. recently developed a robust algorithm to correct RT shifts in GC × GC chromatograms. However there are important\ndifferences in data format and data size between uni-variant detectors such as flame ionisation detector (FID) and multi-variant detectors such as mass spectrometry. In the present study, we adapted the Gros algorithm for GC × GC coupled to high resolution time-of-flight mass spectrometry (HRTOFMS), which is a powerful detector that allows the user to assign compounds by accurate mass spectra. The algorithm is based on user-selected reference peaks that correspond to peaks of identical analytes, identified by the user on both a reference chromatogram and a target chromatogram. The adapted algorithm estimates the shift of each pixel in the target chromatogram relative to the reference. Spectra and signal intensities of each pixel are then estimated by bilinear interpolation to maintain a regular grid of pixels. The algorithm was implemented in MATLAB for 64-bit computers and RT shift correction completes within 10-20 minutes. For evaluating the performance of the algorithm, we analyzed a sediment sample by GC × GC- HRTOFMS with two slightly different oven temperature programs to induce RT shifted chromatograms. By using reference peaks which consist of spiked internal standards and identifiable compounds in both reference and target chromatograms, the artificially shifted chromatograms were aligned based on the reference chromatogram, retaining accurate mass information without generating skewed chromatogram regions. The developed method will be useful for the initial conditioning of the data for more accurate automatic processing, such as peak identification and quantification in GC × GC- HRTOFMS.

TU097

Recursive data processing for quantitative non-target screening in GC × GC-HRTOFMS

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Tens of thousands of chemicals are supposed to exist in the environment. Recent development of mass spectrometer allows us to obtain full spectra of the compounds in analytical samples with accurate mass basis. Comprehensive two-dimensional gas chromatography coupled to high resolution time-of-flight mass spectrometry (GC × GC-HRTOFMS) is the powerful instrument to analyze complex mixtures, separating the mixture by different polarity of capillary columns and scanning full spectra of fragment ions of the compounds with accurate mass basis. However, there are still difficulties to clarify all the contents in the sample, because huge amounts of peak with accurate mass spectra are detected within a run and it takes a lot of labor and time costs to assigning them. In this study, we developed a semi-automatic procedure of recursive data processing for quantitative non-target screening in GC × GC-HRTOFMS. The procedure starts from the analytical data of environmental samples without prior knowledge. The watershed based peak picking in total ion chromatogram of GC × GC followed by spectrum deconvolution for separation of coelution proceeded. The separated and clean-up spectra are all listed in a peak table with its detected retention time values in first GC and second GC (RT1 and RT2), then, automatic spectrum search by NIST MS search is done. The peak with match factor (in simple similarity) less than 900 are filtered out from the list. Duplicated entries within certain RT (RT1 < 1 min, RT2 < 0.5 sec) are also removed. The created database which includes compound-assigned high resolution spectra, RT1 and RT2 are prepared through the process. The data of the samples were recursively processed for identifying and quantifying peak using T-SEN tool, which is reported as related topic in the conference, and the created database. In this approach, the database has chance to expand itself along the increase of the number of the learning sample. Around 300,000 deconvoluted peaks were extracted from 50 samples of river water in the Tokyo bay basin, then, the peaks which meet the above-mentioned criteria were assigned and included in the created database as "prospective" compounds for the further analysis by T-SEN. This approach showed a high-potential for non-target screening of the sample with quantitative information.

TU098

The analysis of new and emerging halogenated organic pollutants in the Great Lakes using passive sampling and comprehensive Two Dimensional Gas Chromatography and High Resolution Mass Spectrometry

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Halogenated organic chemicals (HOCs) are a broad class or organic chemicals that have been and are, used extensively in numerous industrial and commercial applications. As a consequence of this many HOCs are now ubiquitous global pollutants. Due to this many HOCs (e.g. PCBs, PBDEs etc) have been subjected to increasing governmental regulation and have now been phased out of production. As a consequence of this, and changes in technology a whole raft of new and emerging HOCs (eHOCs) have begun to become more widely used. Thus there is a growing need for information concerning their entry and distribution into the environment. The aim of this study was to examine the occurrence of a wide range of these chemicals in the Great Lakes with a view to elucidating their sources and loadings to the environment. The Great Lakes are a collection of extremely large freshwater lakes situated around the Unites States-Canada border in Northeastern North America. This was achieved by systematically deploying a network of low density polyethylene (LDPE) passive samples around the lakes and their major tributaries. These samples were then analysed using a range of analytical techniques including comprehensive Two Dimensional Gas Chromatography (GCxGC) and High Resolution Mass Spectrometry (HR-MS). The identity of unknown pollutants was ascertained using ultra high resolution Fourier Transform mass spectrometry (FTMS). Results from this work revealed significant inputs of a number of eHOCs such as 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), Decabromodiphenylethane (DBDPE) and Hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO) to theGreat Lakes. This analysis also revealed the presence of a number of new HOCs that have not previously been reported in the literature.

TU099

Screening for new compounds in complex matrices using two dimensional gas chromatography (GCxGC) coupled to inductively coupled plasma mass spectrometry (ICP-MS)

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Polyhalogenated compounds (PHCs) are widely used in our society and are frequently both widespread and persistent in the environment. As well, many PHCs are produced naturally, particularly in the marine environment, thus contributing to the complexity of PHC mixtures in samples. Recent studies predict the potential for unmeasured compounds in the environment with some of these compounds present at very low concentrations or hidden in complex matrices. Determining PHCs in samples is usually performed using gas chromatography (GC)/ electron ionization (EI)-mass spectrometry (MS) or GC/electron capture negative ion (ECNI)-MS. For some halogen compounds such as those containing iodine, current GC/MS methods are challenged due to interference by aliphatic hydrocarbons that are common in sample extracts. Therefore, a more sensitive and selective methods would be useful to screen for new compounds. Multidimensional separation technologies such as two dimensional GC (GCxGC) are powerful tools enhancing analytical selectivity and increase resolving power allowing for the identification larger number of chemicals. Additionally, ICP-MS, compared to GC/EI-MS, is selective and nearly interference-free thus is a promising alternative detector for determining chlorine, bromine, iodine, and phosphorous-containing compounds in complex samples at trace level concentrations. Here we explore the feasibility of ICP-MS coupled to GCxGC analysis for the selective determination of PHCs and organophosphorous compounds. Sample matrices examined included house dust, mussel tissue, and sponge tissue. Coupling GCxGC to ICP/MS was successful and results indicate the presence of many additional PHC compounds separated in two dimensional chromatographic space relative to more conventional GC/MS techniques. Of particular interest was the detection of numerous iodine-containing compounds in the matrices, particularly house dust and sponge tissue. Work is currently underway in an attempt to identify the newly detected PHC and phosphorous compounds.

TU100

Optimizing a solid phase extraction method for tributyltin using a design of experiment and quantification with ID-GC-ICP-MS

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The widespread use of organotin compounds (OTC), used as pesticides, antifouling coatings and PVC stabilizers, results in an extensive release into the environment. OTCs show toxic effects even in trace levels. The public concern is focused on the toxic and endocrine disruptive tributyltin (TBT) and its metabolites. In 2000 the European Waterframework Directive (WFD 2000/06/EC) was remitted to standardize the monitoring of aquatic ecosystems and ground waters within the EU. Furthermore, the improvement of water quality and the sustainable usage of water are main objectives of the WFD. Within the scope of the project "Traceable measurements for monitoring critical pollutants under the European Water Framework Directive" of the European Metrology Research Programme (EMRP) quantitative methods for priority hazardous substances, like TBT, will be developed. Standardized methods for TBT in water at the environmental quality standard level (EQS) 0.2 ng L^{-1} do not currently exist. For TBT a limit of quantification of 0.06 ng L^{-1} is specified for the whole water body. A sensitive analytical method is required to achieve this specification. Part of the project concerns the evaluation of different extraction methods like liquid liquid extraction (LLE), solid phase extraction (SPE) and solid phase microextraction (SPME), as best preconcentration methods for TBT quantification at EQS levels. SPE is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture, according to their physical and chemical properties. SPE is used to concentrate and purify samples for analysis and is achieved through the interaction of three components: the sorbent, the analyte and the solvent. For an extensive method development considering all variables the number of experiments will rise to a high number. A statistical approach (Design of experiment DOE) for SPE method development is presented, permitting the evaluation of the most promising extraction parameters by performing only a minimal amount of experiments. Extracts are analyzed using GC-ICP-MS and are quantified by isotope dilution (ID). Optimized SPE parameters for TBT are presented and compared to other extraction techniques.

TU101

Application of Chemical & Nuclear Techniques to study the Groundwater Contamination at Delhi India

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Abstract In order to investigate the effect of contamination of the Najafgarh drain basin area at Delhi on the quality of groundwater in lined and un-lined track of the drain, a systematic program was taken up to map the chemical characteristics and isotope signatures of groundwater. Since shallow aquifer is highest vulnerable to surface pollution, 102 groundwater water samples were collected using hand-pumps at a depth in range of 10.66 to 36.57m and jet pump ranging from 60 to 80m in close proximity to the drain reach during the year 2010-2012 and analyzed for 17 different parameters covering major ions and stable isotopes by following the "Standard Methods of Examination of Water and Wastewater" 21st edition, Washington DC, 2005. The Isotopic signatures of water sample are reported in the conventional δ (‰) notation as a deviation with respect to the isotopic ratio of reference Vienna Standard Mean Ocean Water (V-SMOW) for water. Multi-constituent mixing models of pollutants and isotopic signature of groundwater provided a unique understanding of pollutants dynamics in groundwater. Isotopic data reveals that the aquifer in the studied area does not constitute a homogeneous system in its lateral extent. The groundwater recharge variation from location to location and pumping induced groundwater intermixing through different flow pathways results in wide range of spatial variations in stable isotope (^{18}O) signature of groundwater with $\delta^{18}O$ values as depleted -10.2664 ‰ to -1.248 ‰ during 2010-11; from -8.5872 ‰ to -2.736 ‰ in the groundwater of Najafgarh drain basin area during 2011-12, and -8.979 ‰ to -1.248 ‰ during 2010-11; from -8.131‰ to -2.736‰ in the groundwater of south-western part of Najafgarh drain basin area during 2011-12. The study established that groundwater has become more vulnerable to contamination and isotope studies should be conducted as part of comprehensive hydro chemical investigation of ground water vulnerability. There are indications of pollutants transport from the western, northwestern and southwestern areas to the urbanized and overexploited parts.

Ecological Consequences of Exposure to Pharmaceuticals: From the Laboratory to the Field (P)

TU102

Selection of the dirty dozen of pharmaceuticals of greatest concern to the aquatic environment.

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Aquatic organisms are exposed to many thousands of chemicals discharged by the human population in developed nations. Many of these chemicals are considered disruptive or harmful to aquatic wildlife and the literature on possible targets, effects, species and chemicals grows daily. But given that our time and resources are not infinite, can we identify which of these chemicals currently represent the greatest threat to our wildlife? An area of increasing concern is the pharmaceuticals, where once again the struggle is to identify which of them really represent the greatest threat. In this study we compiled a short list of the most important pharmaceuticals of concern by reviewing several different approaches to risk ranking. These included rankings based on prescription data, tonnage used, concentrations in the environment, PEC/PNEC ratios, PBT, and human to fish plasma therapeutic dose approaches. From these separate ranking approaches we compiled our own 'dirty dozen' list of pharmaceuticals which were most frequently cited as being of concern. These were Ibuprofen, Diclofenac, Paracetamol, Carbamazepine, Naproxen, EthinylEstradiol, Propranolol, Atenolol, Citolopram, Sulfamethoxazole, Fluoxetine and Estradiol. For each pharmaceutical the complete literature on effect concentrations was compiled and compared with measured or predicted river concentrations in the UK. The 12 pharmaceuticals were then ranked in order of concern, either by comparing the median effect and river concentrations, or the lower 5%ile effect and median river concentrations. The relative risk of this group of pharmaceuticals was compared with those of metals and some persistent organic pollutants.

TU103

Screening of hot spots of emerging pollutants in soil, ground water and surface water in The Netherlands: breaking the vicious cycle

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Emerging pollutants are well known from the aquatic sciences, but often less studied in the soil environment. There are, however, many pathways by which emerging pollutants may reach the soil and consequently the ground water by infiltration or the surface water by leaching. In 2012 a pilot project was conducted with the objective to investigate the presence and potential risks of pharmaceuticals (veterinary and human) and steroidal hormones in the Dutch soil environment. Pilot studies focussed on four scenarios of contamination that were chosen in collaboration with stakeholders such as the Dutch Soil Network SKB, two water boards, two regional authorities (provinces) and the Soil Protection Technical Commitee TCB, all of which also contributed financially to the study. The selected contamination scenarios were (1) parasiticide use in equestrian sports facilities, (2) dumping on soil of sludge from collected near sewer overflows, (3) soil infiltration of sewerage from overflows and leaking sewers, and (4) application of pig slurry concentrates on arable fields as an alternative for artificial fertilizers. In general the results indicated that the sources were contaminated by pharmaceuticals and hormones as expected (horse dung, sludge, sewerage, slurry concentrate), but that application to the soil did in most cases not result in measurable contamination of ground water and surface water during a monitoring period of 2 months. The highest risks is associated with a sewer overflow that empties directly into a pond. Human pharmaceuticals that were detected in a drinking water well close to a leaking sewer represent an undesired presence, but not a direct risk to people. Measured concentrations of the parasiticide ivermectin in dung at the horse riding facilities are high enough to kill dung insects and the human pharmaceuticals present in the sludge from a monitored stream may affect aquatic life in the sediment.

TU104

Can ocean acidification affect the susceptibility to emerging contaminants in marine bivalve early-life stages?

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Marine organisms are exposed to a wide range of anthropogenic substances, many of them considered as emerging contaminants due to their growing production and not well-known environmental impact. Among emerging contaminants, pharmaceuticals are cause for increasing concern, being bioactive substances widely used in both human and veterinary medicine. Climate changes, such as ocean acidification, could have a powerful effect on pharmaceuticals by altering their environmental behaviour and exposure pathways, thus resulting in an increased toxicity. Furthermore, shifts in environmental parameters could alter marine organism susceptibility to these compounds. In this context, the combined effects of seawater acidification, as predicted in climate change scenarios, and diclofenac, a non-steroidal anti-inflammatory drug, were investigated for the first time in larvae of the clam *Venerupis philippinarum*. An experimental flow-through system was used to carry out a 96 hours exposure of clam larvae. Fertilized eggs, obtained by adults (15 males and 15 females) kept at pH 8.1,22°Cand 32 salinity during gametogenesis, were exposed to two different levels of pH (8.1 and 7.7) combined with two concentrations of diclofenac (0 and 0.5 μg/L). Three replicate tanks for each experimental condition were set up. Larval mortality and growth were daily checked and recorded. The experiment was stopped as soon as in one of the treatment tank 50% mortality was reached. At the end of the experiment, pools of larvae were constituted to assess oxidative stress (CAT activity) and lipid peroxidation (TBARS assay). Low pH significantly reduced shell length and height and altered their ratio. Diclofenac significantly decreased shell length only, the reduction being higher at pH 7.7 than at pH 8.1. A strong tissue prolapse outside the shell was observed in larvae kept at pH 7.7. About 50% mortality was observed in larvae exposed to pH 7.7, both with and without diclofenac, while mortality was lower than 10% in controls kept at pH 8.1. pH significantly increased CAT activity in larvae kept at pH 7.7 for 96 hours. No significant difference in lipid peroxidation was found among treatments. This study demonstrated that seawater acidification negatively influenced susceptibility of *V. philippinarum* larvae to environmentally relevant concentrations of diclofenac, mostly by altering shell growth.

TU105

Environmental Risk Assessment for Aquatic Organisms Exposed to Tamiflu Use under Seasonal Influenza and Pandemic Conditions

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There is evidence both from field observations and experimental studies of significant correlations between increased Tamiflu concentrations in sewage treatment plant (STP) effluents and receiving river waters and influenza epidemic or pandemic conditions. The purpose of this work was to assess the potential exposure risk of aquatic organisms and environmental hazards posed by antiviral drug Tamiflu use under seasonal influenza and pandemic conditions in Taiwan. An ecotoxicological model with an epidemiological scheme was employed to compute Tamiflu residues and treatment dosage. A probabilistic risk assessment model was used to estimate risks posed by environmentally relevant hazards. We found that (i) the average predicted environmental concentration (PEC) of Tamiflu residues under a pandemic condition was nearly 36 µg L^{-1}, posing no significant threat on algae yield and growth; (ii) the estimated effect concentration affecting 5% (EC5) of Tamiflu residues are 3.52 (95% CI: 2.61 – 5.39) and 25 (11 – 97) mg L^{-1} for algal yield and growth rate inhibitions, respectively; and (iii) the estimated acute-to-chronic ratio (ACR) is ~57 for algal yield inhibition during Tamiflu use under seasonal influenza and pandemic conditions. Our results also showed that there were no significant risks (risk quotient (RQ) < 1) for daphnid and zebrafish when Tamflu residues removal efficiency in STPs is greater than or equal to nearly 60%. Thus, we suggest that tertiary treatment (e.g., ozonation) need to be taken into account in sewage treatment process under an influenza pandemic condition. We concluded that, from a long-term ecological hazard point of view, Tamiflu use during pandemic is alarming. Our study could lend a contribution to ecotoxicological research on assessing how anti-influenza drug residues frequently found in aquatic systems may have a direct or indirect impact on growth, fecundity, and survival of aquatic organisms.

TU106

Investigations of Photochemical Degradates of Pharmaceuticals in Water Samples

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The twentieth century saw a growth in the development, availability, and use of pharmaceuticals to treat and cure disease. As a result, the increasing release of these pharmaceuticals into the aquatic environment is a now a twenty-first century problem of worldwide concern. Assessing the environmental fate of these contaminants, including processes such as biodegradation, soil sorption, and solar photodegradation, requires an interdisciplinary approach. Unfortunately, some of these natural processes result not in removal of the contaminants but rather chemical transformation of the pharmaceutical into structurally similar compounds that are both more persistent and more toxic to the aquatic environment. In this work, HPLC and LC-MS were used to identify and monitor the phototransformation products of several PhACs with a focus on those with chemical structures suggesting a potential for environmental persistence and/or ecotoxicity. Investigations to assess the environmental fate of these degradates, including the toxicity of some of these compounds to amphibians commonly found in the South Carolina Lowcountry, will be described.

TU107

Managing the quality of fish and shellfish immune capacities after diclofenac exposure in mesocosms.

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Diclofenac, one of the most prevalent pharmaceutical in surface waters, is a non-steroidal anti-inflammatory drug taken to reduce inflammation and pain. Many authors have shown that environmental exposure of fish and shellfish to diclofenac provokes adverse effects in various organs, compromising animal health and

affecting their population dynamics. The use of artificial aquatic ecosystems constitutes a good alternative approach, compared to laboratory or field study, to assess environmental risk. In fact, mesocosms provide a link between field studies (natural environments, without replication) and controlled laboratory experiments (artificial conditions, with replication). In the present work, we want to determine how adult fish (three-spined stickleback, *Gasterosteus aculeatus*) and shellfish (zebra mussel, *Dresseina polymorpha*) might react after 3 and/or 6 months of exposure to 0, 0.1, 1 and 10 μg of diclofenac/L. Whatever species used, few effects on immune parameters were shown. In fish, leucocyte mortality tends to increase and respiratory burst and phagocytosis activity tend to decrease in contaminated mesocosms. The present data were in contradiction with many laboratory studies which detected modulation of immune parameters after a few days of exposure to environmental concentrations. These results suggest a great adaptability of animals to contaminant. In fact, in zebra mussel, 3 months of exposure induced an important haemocyte mortality and cellular percentage destabilization whereas no effect was detected at 6 months. Nevertheless, to verify real impact on fish and shellfish health, these data must also be compared to other biomarker responses and to population structure. For instance, at the highest concentration in stickleback populations, a significant decrease of the juvenile recruitment was observed. It could be due to both higher larval and adult mortality than in control conditions. In consequence, the fish length distribution at this concentration was significantly impacted.

TU108

Transcriptome dynamics in Hydra after exposed to three pain relievers

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Pharmaceuticals and Personal Care Products (PPCPs) are substances used by individuals for personal health or cosmetic reasons and used by agribusiness to enhance growth or health of livestock. Among them, the pharmaceuticals that we take are not entirely absorbed by our bodies, and are excreted and passed into wastewater and surface water. In this study, the transcriptional responses against to exposure of three pain relievers, Diclofenac, Naproxen, and Ibuprofen, in *Hydra* were evaluated. For all three drugs, the median lethal concentrations of the animals (LC_{50}) were determined. To define the repertoire of *Hydra* genes responding to acute exposure of three pain relievers, transcriptome dynamics were examined in a series of exposure time (6 h, 24 h and 48 h) by using 17 K Hydra Expressed Gene Microarray (HEGEM) which contains about 17,000 singletons of hydra genes. The gene ontology analysis was carried out for the differentially expressed genes after exposed to the three kinds of pain relievers.

TU109

Evidence of neurotoxic effects of caffeine and ibuprofen on Corbicula fluminea

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The possible effects of pharmaceuticals on aquatic life are not well understood. The constant release of these substances to aquatic environment even at low concentrations ($ng{\cdot}L^{-1}$ to$\mu g{\cdot}L^{-1}$) may expose biota to unknown chronic effects. The aim of this study was to evaluate neurotoxic responses of the fresh water clam *C. fluminea* after exposure to different concentrations of caffeine (stimulant) and ibuprofen (anti-inflammatory) under laboratory conditions. During the assay water was spiked every two days with caffeine (0; 0.1; 5; 15; 50 $\mu g{\cdot}L^{-1}$) and ibuprofen, (0; 0.1; 5; 10; 50 $\mu g{\cdot}L^{-1}$) during 28 days. Stock solutions of pharmaceuticals were prepared in DMSO (0.001% v/v) Dopamine (DOP) levels, Arachidonic Acid Clyclooxigenase activity (COX), Monoamine Oxidase activity(MAO), Mitochondrial Electron Transport (MET), Total Lipids (TLP), Vitellogenin (VTG) concentration, and Energy Consumption (MET/TLP) were determined in gonad tissues. Results showed an increasing dose-dependence of biomarker responses after exposure to caffeine ($p < 0.05$) except for VTG concentration, which decreased when increasing the drug concentration ($p < 0.05$). Neurotoxic effects included significant increase in DOP (5, 15, 50 $\mu g{\cdot}L^{-1}$), COX (5 $\mu g{\cdot}L^{-1}$), MET (50 $\mu g{\cdot}L^{-1}$) and a decrease in MAO (5 $\mu g{\cdot}L^{-1}$). Regarding Ibuprofen exposure, it was observed a decrease in VTG and DOP levels when increasing concentration of this drug ($p < 0.05$). Nevertheless, and increasing dose-dependence was also observed in the rest of the biomarkers tested ($p < 0.05$). In this case, neurotoxic effects included significant increase in COX (50 $\mu g{\cdot}L^{-1}$), MET (50 $\mu g{\cdot}L^{-1}$). MAO did not show differences with control clams. The performed research work revealed that environmental concentrations of CAF and IBU induced neurotoxic effects. On the other hand, environmental concentrations of caffeine and Ibuprofen are capable to induce neuroendocrine effects in *C. fluminea* when decreasing VTG levels in gonad tissues. The selected sublethal responses constituted suitable biomarkers in the assessment of pharmaceutical environmental risk in aquatic environment using *C. fluminea* as bioindicator species.

TU110

Acetaminophen ecotoxicity in a changing environment: oxidative effects in the edible clam Venerupis philippinarum under different conditions of salinity

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The contamination of the aquatic environment by pharmaceutical drugs has been considered an emerging issue in Ecotoxicology. Considerable levels of pharmaceutical drugs have been reported in surface waters, but also in seawater. One of the most used pharmaceutical drugs is acetaminophen (paracetamol) due to its antipyretic and analgesic properties. However, paracetamol exposure can cause adverse effects, especially when administered in over dosage, by the formation of toxic metabolites that end up activating oxidative pathways. Hence, the toxicity of acetaminophen results in multiple effects, including protein denaturation, lipid peroxidation and DNA damage. Considering the ubiquitous presence of acetaminophen in the majority of aquatic environmental matrices, it is important to assess its toxicity, mainly by quantification of the oxidative effects it may cause on non-target organisms. Additionally, biological responses (including the antioxidant defense organisms of most species) can be modulated by abiotic factors, including those challenged by climatic changes; in estuaries, and given the occurrence of extreme events, salinity is an important factor to consider when assessing toxicological effects on biota. The intention of the current study was to illustrate the toxic effects (in terms of the antioxidant defense system; activities of GSTs isoenzymes and glutathione reductase activities; oxidative damage: lipoperoxidation) of acetaminophen in the clam species *Venerupis philippinarum*, under distinct values of salinity. Water salinity influenced the response of the clams to different acetaminophen concentrations, showing the importance of studying physiological traits under realistic test conditions, which are likely to vary in great extent as a result of climate change.

TU111

Ecotoxicity of four non-steroidal anti-inflammatory pharmaceutical pollutants on germination and growth of Lactuca sativa seeds

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Pharmaceuticals are members of a group of chemicals named micropollutants of emerging concern as increasing evidence suggest their ubiquity in the environment and potential adverse effects on non target organisms and humans. Pharmaceuticals not completely degraded in the sewage treatment plant are being discharged in treated effluents but often into sewages ludge. As a result, considerable amounts of these sustances remain in treated sewage sludges (commonly termed biosolids). Following the soil application of reclaimed water and biosolids, pharmaceutical residues can enter in the terrestrial environment. Although an abundant literature on the ecotoxicity of pharmaceutical residues in water on non target organism is increasing, there is very little available information with regard the effects of pharmaceuticals upon the terrestrial environment, and particularly to terrestrial organisms. Compounds with strong sorption and recalcitrant to degradation remain in surface soils and have the potential to be subsequently uptaken by plants. The aim of this study is to assess the ecotoxicological risk of three non steroid anti-inflamatory drugs (NSAIDs); Ibuprofen, diclofenac, salicylic acid and one analgesic amine: paracetamol; all of them found within highest concentration range in environment. In order to conduct this study, a standardized phytotoxicity method was followed along with seeds of lettuce (Lactuca sativa L.). In addition, chemical analysis of artificial soils was completed to quantify the persistence of the four pharmaceuticals throughout time and verify exposure concentrations. Tested products showed a significant ecotoxicity and persistence in soil. The study of inhibition in the elongation of the radicle and hypocotyl of the seeds have provided additional information about sublethal effects. These preliminary results suggest that the ecological impact of these pharmaceuticals residues in soil must be taken into consideration.

TU112

Dealing with confounding factor: Alterations of Dreissena polymorpha gonads exposed to a pharmaceutical pollutant (diclofenac) or to nutritive stress

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The zebra mussel *Dreissena polymorpha* is a fresh water bivalve considered as

suitable monitoring organisms because of it abundance, wide distribution and interesting responsiveness to pollutant. However it also presents a sensibility to variation of environmental parameters, particularly to modification of the nutritive capacity of the environment. So the nutritional state of mussels may constitute a confounding factor in ecotoxicological survey. The non-steroidal anti-inflammatory drug diclofenac is one of the most used drug in France (10 tons per year) and it is part of the three pharmaceuticals proposed for the Water Framework Directive's priority list of pollutant in surface waters. Few data exist on its effect on bivalves. So the goal of this study was to assess the impact of this drug on reproductive apparatus of the zebra mussel. In this aim mesocosm approach was developed with 12 artificial canals and a flux of water containing 0, 0.1, 1 or 10 μg/l of diclofenac in triplicate. The histopathological alterations eventually induced were analyzed in 10 to 20 mussels after 0, 46, 64 and 160 days. However, it appeared that the diclofenac have an impact on macrophyte and periphyton biomass and distribution. To analyze the effect of nutritive stress on gonadic structure of the bivalve, zebra mussels were placed during 150 days in aquarium and fed two time per week by controlled alga volume (*Scenedesmus obliquus* and *chlorella*) in duplicate: 100 000 cells (*ad libitum* diet called 100%), 10 000 cells (10 %) or to 0 alga (0%). Observed ovary histopathological alterations induced by diclofenac (follicle atresie, oocyte necrosis, perifollicular fibrosis, decrease in mature ovocyte production) revealed the gonadic toxicity of this drug. The nutritive stress (10 %) and the total food deprivation (0%) also produced important alterations of the mussel ovary development (follicle atresie, reduction of mature oocyte size). However in the mesocosm experiment the mussel diet was modified but not severally reduced. Moreover the gonadic alterations observed with diclophenac were not similar to these obtained with nutritive deprivation. So it was possible to conclude that the gonadic alterations observed depended mostly of diclofenac direct toxic impact. These results were discussed with amounts of energetic reserves, activity of digestive enzymes and foot protein expression in order to better appreciate the way that *Dreissena polymorpha* adapt to nutritive deprivation.

TU113

Assessment of direct and indirect photodegradation of selected drugs acting on the central nervous system

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Drugs acting on the central nervous system (CNS), especially antidepressants have a very high growth dynamics of consumption. The widespread use of these compounds has lead to their detection in surface waters and ground waters. In recent years, the occurrence and fate of pharmaceutically active compounds (PhACs) in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry. Solar radiation is the main abiotic factor influencing the distribution of chemical compounds present in the environment. Many drug substances and drug products are found to be decomposed under exposure to light, but the practical consequences will not necessarily be the same in all cases.The aim of the research was a comprehensive evaluation of photodegradation of selected drugs acting on the central nervous system. Aqueous solutions of drugs were irradiated in sunlight simulator without (direct photodegradation) and with the addition of humic acid (indirect photodegradation). The samples were tested using the bioassay and physicochemical methods. Toxicity of the samples were evaluated with standard organisms, from bacteria (Microtox), through protozoa (Spirotox) to crustaceans (Daphtoxkit F™ Magna, Daphnid, Thamnotoxkit F). At the same time the concentration of the parent substances was monitored using HPLC. Mass spectrometry was used to identify the photoproducts formed. Most of the pharmaceuticals tested were toxic in the bioassays. Their photoproducts were also toxic to test-bionts, as the toxicity of the samples decreased slower than the concentration of the parent compounds. Moreover, the sensitivity of the assays applied depended not only on the drug, but also on the photodegradation process. The addition of humic acid in the indirect photodegradation influenced the photodegradation ratio evaluated with chemical and biology analysis.

TU114

Toxic effects of carbamazepine on aquatic insect larvae

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Urban wastewaters contain among others a large variety of pharmaceutically active compounds and its metabolites. However, since the ubiquity of these compounds are only insufficiently degraded in wastewater treatment plants they are emitted into surface waters. After entering the aquatic environment freshwater species are exposed to an unknown, but probably very large number of compounds. Of particular concern is the long-term exposure of these aquatic organisms to low doses of pharmaceuticals. In order to elucidate these ecological impacts, Carbamazepine (CBZ) serves as a candidate for an environmentally persistent pharmaceutical. CBZ is an antiepileptic drug, which is only unsufficiently removed during sewage treatment. Due to known effects of CBZ on *Chironomus riparius* larvae, these outcomes will be reinvestigated more precisely by looking at the sensitivity of different larval stages. Moreover the influence of CBZ on *Culex pipiens f molestus* will be examined as another representative within the suborder of midges. A 28 day sediment test according to OECD guideline 218 was conducted with *C. riparius*. The experiment was carried out using four different larval stages exposed to spiked sediment. CBZ concentrations were 0.8, 2, 4, and 20 mg/kg dry weight sediment. *C. pipiens f molestus* was exposed to CBZ in a chronic 28 day experiment in the water phase to model their specific habitat. First instar larvae were exposed to the following CBZ concentrations 0.16, 0.32, 0.64, 1.28, 2.56, 5.12 and 10.24 mg/L. These concentrations refer to the measured concentrations in the water phase in Oetken et al.(2005). First experiments indicate an impact of CBZ on *C. riparius* and *C. pipiens f molestus*. Besides the indirect effects on pupation of *C. riparius*, a clear difference occurred regarding the sensitivity between first and fourth larval instars. The sensitive window of exposure for triggering the effect seems to be in the first or second larval stage. *C. pipiens f molestus* also showed a delay in emergence rate starting at a CBZ concentration of 2.56 mg/L. Additional tests with mayflies revealed a high sensitivity of the larvae towards CBZ. Ongoing experiments will focus on the effects of CBZ metabolites and the influence of ozonation on the degradation of those compounds.

TU115

Effects of carbamazepine and fluoxetine to the springtail Folsomia candida under a multigeneration exposure

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The study of the potential pernicious effects of human pharmaceuticals in the environment can be considered incipient in terms of soil organisms, which are likely to be exposed after sewage sludge disposal in soils. This study assessed the effects of carbamazepine (antiepileptic) and fluoxetine (antidepressant), two human pharmaceuticals found in wastewater treatment effluents, biosolids and surface wasters, on the springtail *Folsomia candida*. Organisms were exposed to spiked soil for 3 consecutive generations to assess long-term effects on parameters associated with biotransformation, neurotransmission and peroxidative damage as well as survival and reproduction on each generation. Behaviour was also assessed in terms of light avoidance. Behavioural changes were assessed in the third generation. Results demonstrate that fluoxetine and carbamazepine affected the reproductive capacity and survival of *F. candida,* especially when exposed for three generations to contaminated soil. After the three generational exposures, behaviour was also affected, and organisms from higher concentrations' exposures lost the ability of moving to sheltered areas, comparing to control situations where almost all animals escaped from light. The biochemical endpoints revealed that both pharmaceuticals are able to induce oxidative stress and affect neurotransmission. Overall, results emphasize the relevance of studying generational exposures to pharmaceuticals since some effects may only be detected after three generations as found in this study.

TU116

Presence of carbamazepine in coastal systems: effects on bivalves

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Most pharmaceuticals are not completely degraded after administration, and some of their metabolites and unchanged forms are excreted into the aquatic ecosystem. Thus, the environmental occurrence of pharmaceuticals, especially in aquatic systems, is a source of growing concern due to the possibility of ecotoxicological risks to the inhabiting organisms. In the last decade, researchers have detected a wide variety of pharmaceuticals in the aquatic environment which include carbamazepine (CBZ), an antiepileptic drug. CBZ shows a low degradation rate upon wastewater treatment plants (WWTPs) so it has been detected in WWTPs influents and effluents, surface waters, groundwater and even in drinking waters in concentrations from 0.03 to 6.3 µg/L. Its environmental persistence raises concerns about the potential effects on nontarget organisms. In fact, some authors have been classifying CBZ as "R52/53 Harmful to aquatic organisms and may cause long term adverse effects in the aquatic environment". In light of that, in the present work, we investigated the effects of environmentally relevant concentrations of CBZ in two clam species from Ria de Aveiro (Portugal). The clams *Venerupis decussata* (native) and *V. philippinarum* (invasive)were exposed to CBZ-spiked water at concentrations between 0.03 and 9 µg/L, for 96 hours. The tolerance of both species, to CBZ, was assessed through the analysis of biomarkers that indicate toxic stress, namely, lipid peroxidation (LPO), glutathione S-transferase (GST) activity,

and glycogen content. The mortality rates, of both species, were also assessed. The results obtained showed that, after exposure, none of the treatments induced mortality. Furthermore, CBZ induced slight and similar responses in glycogen levels and GST activity in both species. This trend was also observed for LPO between 0 and 3 μg/L CBZ. However differences compared to the control were noticed at the highest CBZ concentration (9 μg/L CBZ), where *V. philippinarum* increased LPO levels and *V. decussata* decreased. These results indicate that *V. philippinarum* was under oxidative stress while in *V. decussata* theoxidative stress seems to decrease.

TU117

Environmental Risk Assessment of Propranolol

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Propranolol is one of the most widely studied human pharmaceuticals in the context of environmental risk assessment. The original work from Huggett et al (1,2) suggested that the concentrations in the environment may lead to harmful effects in fish. Since then numerous studies have been undertaken by different workers in order to improve understanding of the risks associated with the use of propranolol, including the major EU project ERApharm which looked at B-blockers more generally. There are now many more ecotoxicological studies available on the effects of propranolol on a range of aquatic species, as well as several studies looking at the predicted fate and exposure of propranolol in the environment and numerous papers reporting actual measured concentrations of propranolol in various environmental media. This poster will present a comprehensive summary and analysis of all the published data available for propranolol and an assessment of the environmental risks using traditional and catchment scale approaches. From all the data available, the majority of measured concentrations in the environment are lower than the predicted no-effect concentration (PNEC), providing reassurance that propranolol does not appear to present a significant risk to the environment. 1) Huggett DB, Brooks BW, Peterson B, Foran CM, Schlenk D. Toxicity of select beta adrenergic receptor-blocking pharmaceuticals (B-Blockers) on aquatic organisms. Archives of Environmental Contamination and Toxicology 2002;43(2):229-235. 2) Huggett DB, Khan IA, Foran CM, Schlenk D. Determination of beta-adrenergic receptor blocking pharmaceuticals in united states wastewater effluent. Environmental Pollution 2003 /2;121(2):199-205.

TU118

Assessing the environmental hazard of mixtures of pharmaceuticals: chronic toxicity of fluoxetine and propranolol to the crustacean Daphnia magna

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Widespread occurrence of pharmaceuticals residues (ng/L to microg/L) has been reported in aquatic ecosystems. However, their toxic effects on aquatic biota and environmental risks remain unclear. Generally, the acute toxicity towards non-target organisms has been assessed in laboratory experiments, while chronic toxicity studies have been performed rarely. Of importance appears also the assessment of mixture effects, since pharmaceuticals never occur in waters alone. The aim of the present work is to evaluate chronic toxic response of the crustacean *Daphnia magna* exposed to individual pharmaceuticals and mixtures. We tested fluoxetine, a selective serotonin reuptake inhibitor widely prescribed as antidepressant, and propranolol, a non selective β-adrenergic receptor-blocking agent used to treat hypertension. Chronic reproduction tests were performed according to 211 OECD guidelines. Single chemicals were first tested separately. Toxicity of binary mixtures was then assessed using a fixed ratio experimental design. Five concentrations (0.5, 0.71, 1, 1.41, 2 Toxic Units) and 5 percentages of each substance in the mixture (0, 25, 50, 75 and 100%) were tested for a total of 26 experimental conditions, including the negative control. Six replicates for each treatment were carried out. The conceptual model of Concentration Addition (CA) was adopted in this study, as we assumed that the mixture effect mirrors the sum of the single substances for compounds having similar mode of action. The MixTox model was applied to analyze the experimental results. This tool evaluates if and how observed data deviates from the CA model, and tests if significantly better descriptions of the observed data can be achieved using a set of deviation functions. These functions allow a differentiation between synergism and antagonism, along with deviations based on the dose-level and chemical ratio dependency. EC50 estimated from single chemical tests were 0.74 mg/L for propranolol and 0.24 mg/L for fluoxetine. Results showed a significant deviation from CA model that indicated antagonism between chemicals in chronic mixture tests. These data are in agreement with previous experiments performed in our laboratory on short-term toxicity of fluoxetine and propranolol alone and in different combinations, using the same experimental design.

TU119

Initial microbial ecotoxicity assessment of Thioridazine, Thioridazine 5-Sulfoxide and photolytic mixtures of Thioridazine

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Thioridazine (THI) is a phenothiazine compound which has been extensively used for decades as antipsychotic drug. Recent studies suggest THI as affordable antimicrobial agent for the treatment of intracellular infections caused by multiresistant strains of *Mycobacterium tuberculosis* and *Plasmodium falciparum*. Therefore, THI might become environmentally relevant again, especially in resource-poor countries plagued by endemic infectious diseases. In this study, the microbial ecotoxicity of THI, its mammalian metabolite THI-5-Sulfoxide (THI-5-SO) and photolytic mixtures of THI after treatment with high-pressure Xe lamp (300 nm – 800 nm) was assessed, using a modified luminescent bacteria test for the analysis of acute and chronic effects towards *Vibrio fischeri*. THI caused a significant inhibition of bacterial luminescence emission and cell multiplication at low concentrations (acute luminescence inh.: $EC_{10} = 0.5$ mg L^{-1}, growth inhibition: $EC_{10} = 2.5$ mg L^{-1}). In contrast, THI-5-SO, which is also formed during photolysis of THI, was significantly less active (acute luminescence inh.: $EC_{10} = 23.6$ mg L^{-1}, growth inhibition: $EC_{10} = 56.6$ mg L^{-1}). Within 256 min of irradiation ($[THI]_0 = 50$ mg L^{-1}), THI was almost completely eliminated (97.4% primary elimination) but not fully mineralized (11% carbon elimination). During photolysis, mixture toxicity decreased in a similar trend like the concentration of THI, indicating a significantly lower microbial toxicity for the photolytic mixtures compared to the parent compound. The well-recognized antimicrobial activity of THI was confirmed by these findings. THI showed high antimicrobial activity in the luminescent bacteria test, but it can be easily degraded after administration to less active products by human metabolism and sunlight. This might be especially beneficial in areas with low wastewater treatment standards, because conventional antibiotics are often highly persistent when they reach the environment. Therefore, THI might be considered as a sustainable alternative also from an environmental perspective. To test this hypothesis and for a better understanding of the environmental risk in general, further research on the environmental fate and effects of THI and its PTPs is mandatory. **Acknowledgements:** J. Menz received a scholarship from the Innovations-Inkubator Lüneburg. C. Trautwein received a scholarship from Deutsche Bundesstiftung Umwelt (DBU). M.L. Wilde received a scholarship from CNPq-CsF-Brazil.

TU120

Combined effects of the pharmaceutical sertraline and Microcystis exposure on Daphnia magna

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This study examines the effects of an environmental contaminant (pharmaceutical) in combination with a natural stressor (toxin-producing cyanobacterium) on the freshwater crustacean *Daphnia magna*. We detected a significant interaction between exposure to the selective serotonin reuptake inhibitor (SSRI) sertraline along with exposure to a strain of the cyanobacterium *Microcystis aeruginosa*, which produces both microcystin and cyanopeptolin toxins, using a life history approach. Endpoints examined include juvenile growth rates, dry weights/sizes, C:N:P ratios, microcystin content, population growth rates, and mortality of daphnids. Treatments containing both stressors resulted in a greater impact on time to maturity, juvenile growth rates, and clutch size, known from prior studies to be key factors influencing population growth rate. With increasing anthropogenic impact on the environment, e.g. eutrophication and global climate change, the occurrence of cyanobacterial blooms will likely increase in the future. The concomitant increasing consumption of pharmaceuticals and effluents of domestic wastewater streams into the environment warrants further research into the potential of interactive impacts of anthropogenic and natural stressors on aquatic life.

TU121

Environmentally relevant concentrations of antidepressants affect crustaceans

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The effects of human drugs in aquatic ecosystems have raised increasing concerns, however, very little is known about the effects of antidepressants, such as the Selective Serotonin Re-uptake Inhibitors (SSRIs), the Serotonin-Norepinephrine Re-uptake Inhibitors (SNRIs) and Serotonin Antagonist and Re-uptake Inhibitors

(SARIs). The neurotransmitter serotonin is the main target of many antidepressants. These drugs alter serotonin levels in the synaptic cleft by inhibiting its re-uptake into the presynaptic cell, which result in a increase stimulation. Within the invertebrates, many biological functions including reproduction, maturation, metabolism, moulting as well as behaviour can be under control of serotonin. The effects of serotonin and the antidepressant drug fluoxetine have recently been shown to alter the behaviour of the marine amphipod, *Echinogammarus marinus* at environmentally realistic concentrations (1-100ng/L). Furthermore, several neuropeptides have been demonstrated to be significantly impacted by antidepressants and might induce side-effects on the fitness of crustaceans. The purpose of this ongoing study is currently analysing the effects of a variety of antidepressants on, phototaxis and predator-avoidance-related behaviour and neurological endocrine disruption in *E. marinus*. Behavioural and transcriptional changes in this crustacean were studied when exposed to the most prescribed SSRIs (citalopram, sertraline and fluoxetine), SARI (trazodone) and SNRI (duloxetine). The animals were exposed to these five drugs at environmentally relevant concentrations from 0.001 to 1 µg/L during short-term (1 hour and 1 day) and medium-term (8 days) experiments. The movement of the amphipods was tracked using the behavioural analysis Daniovision during 12 min alternating dark/light conditions. In addition, the recently sequenced transcriptome for *E. marinus* provide us a mine for neurological genes relating to serotonin and neuro-peptides production, transport and breakdown. Subsequently, the gene expression of neuropeptide hormones such as the Crustacean Hyperglycemic Hormone (CHH) and the Moult-Inhibiting Hormone (MIH), and the expressions of genes involved in the serotonin metabolic pathway were quantified in these animals using qPCR. So far, results have revealed significant changes in amphipods behaviour and their neurophysiology following exposure to low and environmentally relevant concentrations of antidepressants.

TU122

Genetic damage, lipid peroxidation and biotransformation enzymes in amphipods Ampelisca brevicornis in relation to pharmaceutical products spiked in sediment: carbamazepine, ibuprofen and propranolol.

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Acute toxicity bioassays determining the mortality rate of amphipods are worldwide recognized as useful tool to evaluate marine ecosystems. Nevertheless, lethal responses may not be suitable for pharmaceuticals ecotoxicology assessment. This study attempts to evaluate the potential chronic responses determined in amphipods *Ampelisca brevicornis,* for the environmental risk assessment of pharmaceutical products associated to sediment samples. Concentrations of carbamazepine (CBZ), ibuprofen (IBU) and propranolol (PRO) (500μgL^{-1}, 50μgL^{-1}, 5μgL^{-1}, 0.5μgL^{-1}, 0.05μgL^{-1}) were spiked in clean sediment samples, including environmental concentrations (underlined). After 10-days of exposure, biomarker responses were determined. Phase I detoxification enzyme activities (ethoxyresorufin *O*-deethylase - EROD, dibenzylfluorescein dealkylase - DBF), Phase II conjugation enzyme activity (glutathione *S*-transferase - GST) and antioxidant enzymes (glutathione peroxidase – GPx, glutathione reductase - GR) were determined as biomarkers of exposure. Lipid peroxidation (LPO) and DNA damage (*strand breaks*) were determined as biomarkers of effect. Significant increase was observed in the detoxification of CBZ and PROP catalyzed by EROD ($p < 0.05$). Nevertheless, DBF showed to be the enzyme catalyzing the pharmaceuticals IBU and PROP ($p < 0.05$). Phase II detoxification system and antioxidants activity showed higher values compared with control after exposure to CBZ, IBU and PROP ($p < 0.05$). DNA damage and LPO were significantly lower than the control for IBU and PRO ($p < 0.05$). Environmental concentrations of CBZ showed to activate the responses of Phase I (EROD) and II of the detoxification metabolism not causing any effect. Environmental concentrations of IBU and PROP increased all enzymatic activities and LPO, what demonstrates the oxidative stress potential of these substances. DNA damage was not obtained for any of the environmental concentrations of the pharmaceuticals tested. In this study, amphipods were considered as suitable bioindicator for the chronic toxicity assessment of pharmaceutical products associated to sediment samples. This study demonstrates the necessity of evaluating more sensitive responses as detoxification metabolisms and oxidative stress potential, which may affect the biota exposed to pharmaceuticals and the structure of the ecosystems.

TU123

State-of-the-art environmental risk assessment for valsartan

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Recent publications revealed high concentrations of the active pharmaceutical ingredient (API) valsartan in sewage treatment plant (STP) effluent with a maximum and average concentration of 5300 ng/L and 1600 ng/L, respectively measured in samples from 50 large STPs across the US. Taking these concentrations into account a risk assessment based on state-of-the-art environmental toxicity and fate studies following current EU ERA requirements, OECD guidelines and GLP principles is presented. The environmental risk assessment (ERA) includes chronic toxicity data in fish, Daphnia and algae, as well as a toxicity study on sediment-dwelling larvae of *Chironomus riparius*. Additionally, fate data as required by the current EU ERA guideline for human pharmaceuticals are presented. Environmental toxicity and fate data found in the open literature on valsartan are also taken into account to complement the ERA. Based on the negligible chronic toxicity found for valsartan, the ERA concludes that no risk is expected for the environment in spite of the high usage of this API and concurrent high concentrations found in effluent samples.

TU124

Genotoxicity and ecotoxicity screening of photolytic mixtures from the selective β1-receptor blockers Atenolol and Metoprolol

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Advanced oxidation processes like UV-treatment can result in the formation of mixtures with unknown hazards since drugs within the treated waste water can undergo photolytic transformation. Due to their wide usage, the beta blockers Atenolol (ATL) and Metoprolol (MTL) are detected in the ng/l - μg/l range in surface waters and wastewater treatment effluents. Therefore, this study investigated the genotoxicity and basic ecotoxicity of UV-treated mixtures separately for MTL (100mg/l) and ATL (400mg/l). Samples were taken at different irradiation times and the primary elimination of the parent compounds (using HPLC) as well as the degree of mineralization (using dissolved organic carbon, DOC) were analyzed. The photolytic mixtures were investigated with the umuC test (ISO13829) for genotoxicity and the Ames Aqua test (Xenometrix, AG) with TA98 and TA100 (+/- S9) for mutagenicity. For a first hint of the ecotoxicology potential a modified luminescent bacteria test (LBT) able to determine the acute and chronic toxic effects was used. ATL was almost completely eliminated (97.7%±0.8%) after 256min of UV treatment. The elimination of MTL was only 60.9%±1.4% due to the high initial concentration. The mineralization after 256min of ATL and MTL was only 6.3%±0.9% and 5.3%±0.5%, respectively, indicating the presence of transformation products (TPs). No DNA damaging potential using the umuC test and no mutagenic effects in the Ames test were detected at any time points at the lowest dilution level (1:1.5) of photolytic mixtures. In the LBT an increase of acute and chronic toxicity from 0min to 256min of irradiation was observed in the lowest tested dilution (G2) with maximum inhibition of 74% (chronic luminescence inhibition, chronic LI) for MTL and 85% (acute luminescence inhibition) for ATL. The increase of toxicity with irradiation time corresponds to the decrease of the parent compounds and the appearance of TPs. The lowest effective dilution (>20% inhibition) after 256min was determined at the dilutions G10 (30%, chronic LI) and G8 (27%, chronic LI) for mixtures from ATL and MTL, respectively. Based on the results neither mutagenic nor genotoxic effects were observed. However, photolysis resulted to the formation of ecotoxicology relevant photolytic mixtures, but only in environmentally irrelevant concentrations. Therefore, the UV-treated mixtures do not seem to be a hazard for the environment or humans with regard to their environmental concentrations.

TU125

Predicting antibiotic resistance in aquaculture production systems and surrounding environments

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Aquaculture production has been considered as one important source of antibiotic pollution and antibiotic resistance (AR) genes. Assessing the risks associated with antibiotic pollution and AR in aquaculture and surrounding environments is therefore critical to advice sound environmental management and policy and to derive measures aiming at preventing and mitigating AR risks. In this study we build upon previous research on the environmental fate of aquaculture antibiotics and the selective pressure of antibiotic pollution, and introduce a framework to predict and evaluate the development of AR in aquaculture production systems and their adjacent environments. Such risk assessment framework is supported by two pillars. The first one is based on the modelling of antibiotic exposure in aquaculture and environmental compartments such as water and sediments, which in turn is influenced by information regarding the therapeutic dose and physico-chemical properties of the applied antibiotic, and characteristics of the aquaculture production system including management practices (e.g. aquatic species, water and sediment characteristics, effluent discharge). The second pillar is based on assessing the susceptibility of bacterial populations to develop AR. This is done by constructing Species Sensitivity Distributions (SSDs) with breakpoints derived for clinically relevant bacteria collated from different sources worldwide, including

pharmaceutical companies, medical laboratories and hospitals. Our proposed framework mechanistically links antibiotic exposure and bacterial susceptibility data by applying the theory of probabilistic risk assessment, and yields quantitative estimates as regards to the probability that a given antibiotic application results in the development of resistance in environmental bacterial populations and communities. In the current study, such framework was exemplarily used to predict the development of AR caused by the use of 12 antibiotics in an aquaculture pond scenario representing Vietnamese *Pangasius* catfish aquaculture. We conclude that the proposed framework, although still requires further field evaluations, sets the ground for the inclusion of relevant AR endpoints in the prospective risk assessment of aquaculture antibiotics and can be easily extrapolated to other antibiotic pollution scenarios (e.g. environmental discharges from livestock production and waste-water treatment plant effluents).

TU126

Do environmental concentrations of pharmaceuticals and PCPs select for anti-microbial resistance?

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Antibiotic resistant bacteria present a major threat to human health and an ever increasing financial burden on healthcare systems. Pharmaceutical companies are under great pressure to develop new antimicrobial drugs, which quickly become ineffective due to the ability of bacteria to rapidly develop resistance. A growing concern is that not only are we driving evolution of resistance due to inappropriate and excessive use of antibiotics, but also through releasing them into the environment. Antibiotics are used extensively in the clinic and the community, and as therapeutics, prophylactics and growth promoters in farming and aquaculture. They are often not fully metabolised and so can be excreted as active parent compound or transformation products in faeces and urine. These enter the environment via effluent from water and sewage treatment plants, surface run off and the direct application of manure as fertiliser. Degradation rate varies greatly for different antibiotics, but many persist in the environment at low concentrations. The minimal selective concentration (MSC) is the lowest antibiotic concentration at which selection can occur. MSC has been measured in the laboratory at extremely low levels (ng/l) (Gullberg *et al*. 2011), similar to environmental concentrations. Environmental concentrations of other compounds such as biocides may co-select for antibiotic resistance. This allows for an antibiotic resistance gene to be maintained even in the absence of antibiotics. It can occur when the antibiotic resistance gene is located on the same mobile genetic element as a different gene undergoing positive selection – e.g. a gene conferring resistance to a biocide. Genomic libraries were constructed from biocide-contaminated reed bed soil, sewage cake and uncontaminated grassland soil. These were screened on two biocides: benzalkonium chloride (BKC) and cetyltrimethyl ammonium bromide (CTAB). Resistance to either biocide was approximately 40x more frequent (on average) in reed bed and sewage cake clones than in grassland soil populations. Transposon mutagenesis was performed on plasmids extracted from the resistant clones, which allows sequencing of the resistance genes. Finally, biocide resistant clones were screened on a range of antibiotics to determine whether co-selection may be occurring.

TU127

Residual toxicity of sulfamethoxazole transformation products on bioluminescence and growth of vibrio fischeri (24 h)

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Among sulfa drugs, sulfamethoxazole (SMX) is one of the most prominent short-acting sulfonamide antibiotics. It is frequently detected in urban wastewaters and surface waters alike, and a number of recent studies reported on different chemical or biological transformation pathways occurring in the aquatic environment and the (presumably) released transformation products (TPs). However, limited information is available regarding TPs formed during natural transformation processes, including human metabolism. Some of these SMX TPs contain the pharmacophore sulfanilamide moiety and some derivatives incorporate almost the complete SMX parent compound structure. It is thus reasonable to assume that these compounds still can exhibit residual antimicrobial activity. Moreover, the breakdown products which do not include the sulfanilamide group should also be taken into account considering that these can contribute to a residual toxicity through other modes of action. Addressing this issue, the present study aimed at screening 9 TPs of SMX formed during human metabolism, microbial biodegradation, photolysis and hydrolysis, for their bacterial toxicity when considered as individual compounds as well as mixture. As bioassays, bacterial growth inhibition (GI) was selected to evaluate the bacteriostatic mode of action of sulfonamides, while luminescence inhibition (LI) was selected as a very sensitive and rather non-specific endpoint (both using *vibrio fischeri*). Since short-term tests usually fall short of the delayed bacteriostatic effects of antibiotics, a test duration of 24 h was applied. Results showed that LI EC_{50} of SMX derivatives (N-acetyl-, 4-nitro- and N-hydroxy-SMX) are in the range of SMX, while the EC_{50} of TPs formed from SMX cleavage, including sulfanilamide, are approximately two orders of magnitude higher for luminescence inhibition and one order of magnitude higher for growth inhibition. With regard to time-dependent toxicity, LI results revealed that EC_{50} of the SMX derivatives were 17 to 75 times lower after 24 h than after 30 min, while dose-response relationships of the other breakdown products remained unchanged. 3-Amino-5-methyl-isoxazole, which has been reported as a stable TP from biological and photolytic cleavage of the sulfonamide bond, showed no growth inhibitory effects and, LI was observed only in the upper mg/L range. N-hydroxy- and 4-nitro-SMX were found to be more toxic to growth than the parent compound.

TU128

Residues and health risk assessment of quinolones and sulfonamides in cultured fish from Pearl River Delta, China

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Concentrations of six selected veterinary antibiotics (three quinolones and three su lfonamides) in cultured fish samples from the Pearl River Delta, South China, wer e investigated.

The results revealed that quinolones and sulfonamides were widely distributed in t he cultured fishes. The concentrations of total quinolones ranged from 2.5 to 185.7 μg kg-1 wet weight (w. wt)

while the concentrations of total sulfonamides ranged from 0 to 140.5 μg kg-1 w. wt. Higher levels of veterinary antibiotics (VAs) were found in freshwater fishes t han marine fishes.

The eel and bass contained the highest concentrations of total quinolones (185.7 ± 19.9 μg kg-1 w. wt) and total sulfonamides (140.5 ± 12.5 μg kg-1 w. wt) in the m uscle tissue, respectively.

Antibiotic concentrations in some samples exceed the maximum residue limits (10 0 μg kg-1) set by European Commission.

Estimated exposure to sulfonamides in fishes was negligible as the estimated daily intake (EDI) was less than 1% of acceptable daily intake (ADI)

while the EDI (2-6% of the ADI) of total quinolones would pose a human health ri sk. Due to the potential risk of antibiotics on the aquatic environment and human health,

further investigation on the impact of these emerging pollutants is urgently encour aged.

TU129

Non-target effects of ivermectin residues on soil organisms at 4 grassland sites (France, Switzerland, The Netherlands, Canada)

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Numerous field studies have assessed the non-target effects on dung-breeding insects, of residues in dung of cattle treated with veterinary medical products (VMPs). Comparisons across studies are confounded because of variation in methods, products and insects tested. In addition, few studies have considered the effect of residues on soil organisms dwelling beneath dung pats. To assess the value of including soil organisms in future such studies, four studies were performed in parallel in four biogeographic regions (Switzerland, The Netherlands, France, and Canada). Each study used the same methods based on the recommendations of Jochmann et al. (2011). Treatments comprised dung from untreated cattle and from cattle treated 3, 7, 17 and 28 days previously with the VMP, ivermectin. Earthworms (Lumbricidae) and springtails (Collembola) were collected from beneath the dung, from 1 to 12 months after the pats were placed in the field. Three months post-placement, ivermectin concentrations in soil below dung pats were 0.02 to 0.03 and declined to generally < 0.006 mg/kg dw soil on subsequent dates. In samples collected 1 and 3 months post-placement in The Netherlands, fewer earthworms were recovered from soil collected beneath Day 3 and Day 7 dung, relative to controls. Treatment effects on earthworms were detected at the site in Switzerland 3 and 5 months post-placement, but the pattern was unclear. The four study sites each supported diverse communities of springtails, but their abundance was highly variable during the 12-month study. No effect of treatment on springtails was detected at Montpellier. For samples collected 1 to 5 months post-placement at the other sites, effects of treatment were detected on the total number of springtails, the number of individual age groups and on the number of ecological groups. There was no clear relationship between treatment and degree of effect. Results of this study support inclusion of soil organisms in field studies to assess the non-target effects of VMPs. Jochmann, R., W. Blanckenhorn, L.

Bussière, J. Jensen, U. Kryger, J. Lahr, J-P. Lumaret, J. Römbke, K. Wardhaugh, and Floate, K.D. 2011. How to test non-target effects of veterinary pharmaceutical residues in livestock dung in the field. Integrated Environmental Assessment and Management 7:287-296.

TU130

An international ring test to assess effects of ivermectin

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An international field ring test with ivermectin as the model substance was set up with the following questions: (1) Does the use of ivermectin cause any long term effects on dung fauna biodiversity and in particular, how long does the excreted dung stay toxic to the dung fauna in the field? (2) Are there differences in the sensitivity of single model species tested in standard laboratory tests and those found in the field? Are the species used in the laboratory representative for the field? Four parallel studies were conducted in spring (April-May) 2011 in pasture environments on two different continents and in four biogeographical regions using the same overall design: (1) Lethbridge, Alberta, Canada – North American prairie environment, (2) Montpellier, France – European Mediterranean region, (3) Zurich, Switzerland – European Continental region, and (4) Wageningen, The Netherlands – European Atlantic region. Cattle were treated, like they routinely are, with a pour-on formulation of ivermectin at a dose of 0.5 mg ivermectin/kg body weight. Dung was collected shortly before treatment (Day 0) and 3, 7, 14 and 28 days after treatment (in Canada also on Day 56). The dung was used to conduct two parallel experiments at each field site: (1) a 'structural' experiment to investigate the effects of ivermectin on dung fauna populations and biodiversity, and (2) a 'functional' experiment to assess the effect of the treatment on dung degradation. The study demonstrated that regardless of differences in the composition of the principal dung insect groups among the experimental sites and biogeographic regions, ivermectin residues in general strongly reduce the number of dung-decomposing flies and to a lesser extent also dung beetles. However, this had no noticeable impact on the degradation rate of cattle dung, perhaps because decomposition by earthworms and physical processes are more important. Despite considerable variation among the sites, the findings were qualitatively similar, so the study design is robust and hence suitable to evaluate the effects of parasiticides on dung insects under field conditions as required in higher-tier testing for risk assessment. The design should now be incorporated in an OECD Guidance Document.

TU131

Short-term exposure to erythromycin and enrofloxacin can induces alterations in neuronal and oxidative stress parameters of zebrafish

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Pharmaceuticals are essential for the human and animal health being used to treat and/or prevent diseases. Antibiotics (ABs) are the most commonly used pharmaceuticals but due to its high use the ABs often reaches the environment through different pathways. In the same way that pharmaceuticals induce biological effects on treated organisms they might cause effects on non-target aquatic organisms living in contaminated areas. In this study, zebrafish embryos and adults were used to evaluate the toxic effect of two antibiotics, namely erythromycin and enrofloxacin. To do so, short-term toxity tests were performed with each AB separately following OECD guidelines. Five treatments were used 0, 1, 29, 44, 66, 100 mg L^{-1} and the mortality and behavior alterations were recorded daily. After 96h, the tests were ended and the activity of neurological (Acetylcholinesterase – AChE, butyrylcholinesterase – BChE and propionylcholinesterase – PChE) and oxidative stress biomarkers (glutathione-S-transferases – GST, catalase – CAT and lipid peroxidase – LPO) were measured. Both ABs were slightly toxic to zebrafish with LC50 values > 100 mg L^{-1}. However, alterations on biomarkers activity were found in all treatments (LOEC < 1 mg L^{-1}). For both ABs an induction of the neuronal enzymes AChE and BChE were found but only for enrofloxacin an induction of PChE were observed. The oxidative stress enzymes were also highly responsive to ABs exposure. CAT activity was induced in all treatments of erythromycin and enrofloxacin. Moreover, GST activity was induced after erythromycin exposure and lipid peroxidation was only observed in the 100 mg L^{-1} of enrofloxacin. In summary, the results of this study show that a short-term exposure of zebrafish to erythromycin and enrofloxacin can provoke alterations in several neuronal and oxidative stress enzymes.

TU132

Biological and structural changes in rainbow trout (Oncorhynchus mykiss) after long-term exposure to clotrimazole

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Human pharmaceuticals and their metabolites have been detected in the aquatic environment in various concentrations. The aim of this study was to characterize biomarker responses and structural changes in rainbow trout exposed to clotrimazole (CLO). For this purpose, juvenile rainbow trouts were exposed to sub lethal concentrations of clotrimazole (0.01, 1 and 10 μg/L) for 42 days. At days 21 and 42, several hepatic, gill and brain biomarkers were measured including glutathione-S-transferase (GST), lactate dehydrogenase (LDH), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and content of thiobarbituric acid reactive substances (TBARS). Histological assessments of fish tissues (gill, liver, kidney, and gonad) and hematological indices were also assayed. This study showed that not in all cases antioxidants were disrupted in a dose dependence manner. Antioxidant defense enzymes decreased a negative feedback and prevented oxidative damage of lipids during 42 days of chronic exposure. In GST data a clear response was observable after 42 days with an induction of activity in the middle concentrations in liver and gill tissues. Also increased level of LDH activity was in liver tissue during the first 21 days of exposure. The hematological indices suggest that immunologic system was not affected. Another fact is that granulocytes count was increased with time, which may relate to a stress response. Histological changes were most obvious in kidney and testis. Changes observed in the kidney were most prominent in the tubular epithelial cells, showing tubulonephrosis and hyaline droplet degeneration. In testis, the percentage of spermatozoa was reduced, spermatogonia and spermatocytes were increased. Our study demonstrated that CLO caused structural histological and intracellular changes in rainbow trout. *Keywords*: Clotrimazole, Histology, Oxidative stress, Rainbow trout. *Acknowledgement* - This study was supported by the project CENAKVA CZ.1.05/2.1.00/01.0024, the Grant Agency of USB GAJU 087/2013/Z and the Grant Agency of Czech Republic P503/11/1130, The results of the project LO1205 were obtained with a financial support from the MEYS of the CR under the NPU I program.

TU133

Development of an obesogenic test in Daphnia magna

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The increasing concern on emerging contaminants has urged for the need of novel ecotoxicological procedures in Environmental Risk Assessment. Current toxicity assays such as those based on acute and chronic responses may not be sensitive enough to detect emerging effects at low doses. Here data on a new assay developed in *Daphnia magna* juveniles to test obesogenic effects of contaminants is included. The bioassay is based on short term *in vivo* exposures to the tested chemicals and *in vivo* visualization of lipid reserves using the fluorescent dye Nile red. The test was validated with a model pollutant known to be obesogenic, such as tributyltin (TBT), and other anthropogenic contaminants such as triphenyltin (TPT), 4-nonylphenol (NP), bisphenol A (BPA) di-2-ethylhexyl phthalate (DEHP), piryproxyfen (PP) and fenarimol (FEN), as well as natural hormones such as methyl farnesoate (MF) and 20-Hydroxyecdysone (20E). The results obtained indicated that the Nile red bioassay was able to reproduce lipid reserve dynamics in *D. magna* within and between moulting, reproductive events, juvenile and adult stages, food levels and across the tested compounds. TBT, followed in decreasing order of effects by the juvenile hormone (MF), its insecticide homologous (PP), BPA and the ecdysone 20E increased significantly the formation of lipid droplets measured as Nile red fluorescence. Lowest obesogenic effect concentrations occurred at 0.1-1 μg/l for TBT and PP and at 10-100 μg/l for the rest of substances. Mixture combinations of TBT and 20E with the juvenile hormone or its insecticide agonist PP were synergic, whereas those with feniramol were antagonic. Lipidomic analyses performed in lipid extracts from pools of organisms using an ultra-performance liquid chromatography coupled to a time-of-flight mass spectrometer (UPLC-TOF), indicated that observed Nile red fluorescence changes were related to increased levels of di and triacylglicerides. These results indicated that there is a great potential for xenobiotics to disrupt the lipid metabolism in *Daphnia* and that such effects are related with the ecdysone and retinoid acid receptor signalling pathways. Acknowledgement-This project was supported by the Spanish MEC and Advance grant: EU grants CTM2011-30471-C02-01, ERC-2012-AdG-320737. The

Portuguese Foundation for Science and Technology (FCT), supported the doctoral fellowship of Rita Jordão (SFRH/BD/79453/2011).

TU134

Reducing the emissions of CO2 from a therapeutic intervention in patients with rheumatoid arthritis treated with etanercept or adalimumab

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Introduction: Rheumatoid arthritis is a figure that affects quality of life of patients. New and expensive drugs such as etanercept or adalimumab can improve the health status of the patients and their quality of life. These drugs are provided in a Pharmacy Hospital and patients have to go to the hospital travelling by car. In controlled patients it's possible to optimize the therapy and to reduce the consumption of these drugs. Objective: The aim of this study is to estimate the environmental impact of optimization therapy with etanercept or adalimumab in rheumatoid arthritis patients in a year. Methods: Twelve patients with optimization of therapy in a Spanis hospital were included in the study. Data of kilometers and type of vehicles (gasoline, gasoil or bus) were collected. Five patients treated with adalimumab were able to avoid nine trips to carry out medication per patient and year and seven patients treated with etanercept were able to avoid twenty six trips to carry out medication per patient and year. Results: Because of the great extension of the influence zone of the hospital the twelve patients must to recover 1.166,4 kilometers per trip to carry out their medication. Patients treated with the optimization therapy would be able to avoid an amount of 272 trips and 12.962 kilometers per year, so 2.996 kilograms of CO_2 emissions can be reduced. Conclusion: Therapeutics decisions based in optimization of drugs can reduce the consumption of drugs, the trips of the patient to carry out their medication and the CO_2 emissions to the atmosphere.

TU135

Omeprazole exposure induces biochemical and morphological changes in the fish gills.

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Omeprazole (OME) is a drug widely used to inhibit gastric acid secretion disorders. OME is a blockbuster which has become an important environmental pollutant, due to their presence in aquatic systems such as: wastewater-treatment-plant effluents, rivers and lakes, drinking and ground water. This study reports the OME action on the gills of the freshwater teleost fish, *Oreochomis niloticus,* after subchronic exposure (14 d) to 0.0, 0.1, 1, 10 and 100 μg L^{-1} in water. Ions and osmolality were measured in plasma and the activity of Na^+/K^+-ATPase, H^+-ATPase, carbonic anhydrase (CA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) and the levels of reduced glutathione (GSH) and lipid peroxide (LPO) were measured in the gills. The histopathological damage was also evaluated in the gills. A transitory ionic disruption was observed in fish exposed to 10 μg L^{-1} OME by decreasing osmolality and Na^+ values. Na^+/K^+-ATPase and H^+-ATPase activities did not change after OME exposure, but the CA inhibition shows that OME may affect the ionic regulation in fish. OME induced changes in the antioxidant defenses in the gills by increasing the activity of CAT enzyme at 10 and 100 μg L^{-1} OME exposure and the levels of GSH of fish exposed to OME at 1, 10 and 100 μg L^{-1} avoiding the oxidative stress. No severe and irreversible lesions occurred in the gills after OME exposure and the chloride cell hypertrophy provided evidence of a compensatory response to improve ion uptake. The results show that the presence of OME in water may disrupt ion regulation by a mechanism similar to that in the gastric cells, via inhibition of CA activity, activate the antioxidant defenses and, indirectly, may affect the gill respiratory function due to gill epithelial cell responses which increased the water-blood diffusion distance. This mechanism seems to be dependent on the OME concentration in the aquatic environment. Financial support: CAPES, CNPq/INCT-TA Proc. 573949/2008-5 and FAPESP Proc. 2008/05778-1.

TU136

Exposure concentration- and generation-dependent effect of antibiotic tetracycline on D. magna gene expression using Microarray analysis

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In the present study, it is aimed to investigate the concentration- and generation-dependent genetic responses of non-target organism *D. magna* exposed to antibiotic tetracycline. Multi-generational research was investigated to measure the wide gene expression pattern and its change caused by toxicant stresses. Moreover, it was tried to link the change of genetic expression with higher-level stress responses caused by multigenerational exposure. The chronic exposure of daphnids to different concentration of tetracycline resulted in the apparent change of gene expression of *D. magna*. The intensity scatter plot showed that the repressed or induced gene number was elevated with increasing exposure concentration. Overall, the total number of 2-fold changed genes were increased to 527 (0.1 mg L^{-1}), 899 (1.0 mg L^{-1}), and 2148 (10.0 mg L^{-1}). The exposure concentration specific differential gene expression was observed and exposed profile showed various pattern. The change of gene expression over generations caused by multigenerational exposure to tetracycline was also applied. For the results of generation dependent effect of tetracycline on daphnids gene expression, four successive generations of target organism continuously exposed to 1 mg L^{-1} tetracycline was used for microarray analysis. As a result, the multigenerational exposure of tetracycline to daphnids resulted in the change of gene expression. Compared to the control (F0), the total number of 2-fold changed gene were increased to 1856 (F1), 1306 (F2), and 1918 (F3). To identify the biological processes and molecular functions that were repressed or induced in response to tetracycline, Gene Ontology (GO) functional analysis was performed. As a result, chronically exposed tetracycline induced the abnormal expression of genes including various biological processes and molecular functions. For example, a number of genes involved in energy metabolism including lipid metabolic process, carbohydrate metabolic process and proteolysis were significantly responded to chronic tetracycline stress. The expression of *D. magna* gene is expected to be influenced by the exposure concentration of tetracycline and generation number. Also, *D. magna* genes responded to chronic tetracycline exposure can also be categorized into several functional processes in metabolism and genetic information processes. In addition, the genetic change might have a significant relationship with other biological organization levels.

TU137

Fate of propranolol in soils in presence of Cu(II)

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For 20 years, many studies have reported the presence of new compounds, called "emerging pollutants", in wastewater and aquatic environments mainly coming from wastewater treatment plant (WWTP) effluents and soil amendments (sewage sludge, manure, slurry, …). These emerging pollutants include pharmaceuticals whose concentrations can reach up to a few tens μg in surface waters. Their presence can produce damaging effects on ecosystems as well as on human health. It is therefore fundamental to study their fate in the environment. This study is part of the project TrEcoPolEm which aims to obtain a better understanding of the fate and impact of pharmaceuticals in the environment. We present here the study of a beta-blocker, propranolol, which is used to treat heart diseases such as hypertension, cardiac arrhythmias, and other diseases as anxiety and glaucoma. This persistent compound exhibiting a high water solubility is found at the μg/L level in surface waters. Moreover, propranolol is present in sewage sludges and can reach soils through amendments. Its fate in the environment is governed by sorption processes in soils. Adsorption behaviour of pharmaceutical compounds is difficult to be predicted and depends on various parameters such as soil composition, pH, and contact time that we present in this study. Moreover, given such molecules can interact with heavy metals present in the environment, we also report the influence of copper presence on the propranolol fate in soils.

Macro, micro and nanoplastic pollution in the aquatic and terrestrial environments: Sources, fate, exposure and ecological and toxicological impacts (P)

TU138

Relative importance of microplastics as a pathway for the transfer of hydrophobic organic contaminants to marine life

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Environmental risk assessment of microplastics in the marine environment requires the use of sensitive bioaccumulation models based on physiological processes to predict contaminants concentration in the tissues of relevant marine organisms following ingestion of contaminated plastic particles. Hence environmentally relevant scenarios were investigated according to reported contaminant concentrations in seawater considering both low and highly polluted sites, with both low and high amounts of ingested plastic (1 and 5 % ingested plastic particles). In addition, extreme scenarios (50 % ingested plastics) were also investigated to determine the relevance of POP uptake through worst-case plastic ingestion. A comparison with other routes of exposure (i.e. respiratory uptake and dietary uptake) permitted us to determine the extent of the concern of the ingestion of contaminated microplastics compared to other pathways. The benthic invertebrate *A. marina*, a pelagic fish, and a seabird were selected as candidate organisms for our models as they are representing both cold blooded and warm blooded organisms. Internal concentration of DDT, phenanthrene (Phe) and bis-2-ethylhexyl phthalate (DEHP) in *Arenicola marina*, a fish and a seabird were predicted following PVC and polyethylene (PE) dietary uptake using single –compartment models. Different scenarios were taken into account in order to consider environmental relevant and extreme scenarios (1, 5 and 50% ingested plastics for low and high polluted sites). The relative contribution of microplastics for the bioaccumulation of contaminants compared to other pathways of exposure.

TU139

Interaction of microplastics and oceanographic features in the North Atlantic

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Plastic are found in marine habitats and are recognised as an environmental problem. Levels of marine plastic pollution, including microplastics, are largely undocumented in the Atlantic Ocean. A standardised, replicable method is required to understand the distribution and abundance of microplastic pollution. A novel flow-through system of filtering has been developed for simple, cost-effective replicable sampling on research vessels to collect synthetic particles from seawater. Sampling using this filtering system was conducted during two transatlantic passages in 2013, between Galway and Newfoundland aboard Ireland's research vessel, the *R.V. Celtic Explorer*. Non-biological synthetic particles were found in the North Atlantic across a large spatial scale. Particles were assigned to four product type categories: fibres, fragments, industrial pellets and bead scrubbers, and five length categories: < 1mm, 1-2.5mm, 2.5-5mm, 5-10 mm, and >10mm. The results have been combined with oceanographic data to understand whether there is an oceanic influence on the number of particles found, focusing on areas of warm water upwelling. The results from this study indicate that plastic particles are widespread in the surface layer of the Northeast Atlantic Ocean, trends in abundance and distribution will be discussed. This is the first report of the ubiquitous nature of microplastic pollution in the Northeast Atlantic Ocean and highlights the potential for a method to be used as a standardised monitoring protocol.

TU140

Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics: Leaching of hydrophobic chemicals from ingested plastics to digestive fluid

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We analyzed polybrominated diphenyl ethers (PBDEs) in abdominal adipose of oceanic seabirds (short-tailed shearwaters, *Puffinus tenuirostris*) collected in northern North Pacific Ocean. In 4 of 33 birds, we detected higher-brominated congeners (i.e., BDE209 and BDE183), which are not present in the natural prey (pelagic fish) of the birds. The same compounds were present in plastic found in the stomachs of the birds. These data suggest that transfer of additive-derived BDE congeners from ingested plastics to the internal tissue of seabirds. However, transfer mechanism of PBDEs from ingested plastic to biological tissue has been unrevealed. Because of high hydrophobicity of PBDEs, it is thought that leaching of PBDEs from plastics to aqueous solution is insignificant. We studied possibility that oil in the seabird's digestive tract (stomach oil) facilitates leaching of PBDEs from ingested plastics. We determined the leaching amounts of PBDEs from plastic plate to which DecaBDE was industrially compounded. Distilled water, seawater, acidic pepsin aqueous solution, and simulated stomach oil (fish oil) were used leachants. The conditions of leaching experiments are as follows : liquid-to-solid ratio of 1000:1, contact periods : 5 days and 15days, temperature : 20 °C for distilled water, seawater, pepsin solution and 38°C for pepsin solution and fish oil. The leaching amounts of PBDEs to distilled water, seawater, and acidic pepsin solution were trace, i.e., less than 1% of PBDEs content in the plastic plate. In contrast, fish oil (simulated stomach oil) leached 30% of the PBDEs from the plastic plate. Furthermore, leaching experiments by using actual stomach oil taken from seabird (Streaked Shearwater, *Calonectris leucomelas*) were conducted under basically same experimental conditions. The leaching amounts of PBDEs in actual stomach oil were about 10% of PBDEs content in the plastic sample. Our experiments clearly demonstrated that PBDEs in plastic leach to digestive fluid to a great extent in the presence of stomach oil. This could be an important process of the transfer of plastic-derived hydrophobic chemicals from ingested plastics to the tissues of seabirds.

TU141

AhR agonists in beached marine plastic debris in the Pacific Ocean

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In order to obtain a comprehensive chemical risk assessment of marine plastic debris, all chemicals sorbed to or incorporated in plastic which have negative biological effects should be monitored. A well-established approach to this challenge is to use mechanism-specific bioassays. This enables detection of effects caused by a much wider range of chemicals that can be obtained using chemical analysis. In addition, mechanism-specific bioassays can integrate effects of chemical interactions and enable mass balance calculations to explain observed effects. Using the H1L6.1 CALUX bioassay for dioxins, beached plastic samples from the Pacific Ocean were studied for their content of AhR agonists. Collected plastic samples included fragmented plastic pieces below 1 cm diameter, plastic nurdles, plastic caps, nets, and spacers from Japanese oyster farms. Plastic samples were extracted with hexane using ultrasound extraction (3 times 1 hour). The extracts were dissolved in DMSO and tested in concentration series in the H1L6.1 CALUX bioassay for dioxins for 24 and 72 hours. Expressed as bioTEQs after 24 hour exposure, levels of AhR agonists in plastic caps ranged between 10 and 200 pg/g. For spacers, ropes and nets bioTEQ levels ranged between 10 and 100 pg/g. For plastic fragments bio-TEQ levels ranged between 50 and 1500 pg/g and for plastic nurdles the range was between 200 and 3600 pg/g. One plastic nurdle sample from a beach close to Cape Town in South Africa had bio-TEQ levels of 13000 pg/g. After 72 hour exposure, the levels of bio-TEQs in the extracts from plastic nurdles and plastic fragments from Hawaii had decreased to a range from below detection limit to 280 pg/g. These results show that weathered marine plastic debris can accumulate AhR agonists and also dioxin-like compounds in the northern Pacific ocean. The levels accumulated can constitute a risk for marine life ingesting plastics, from fish to birds. A preliminary exposure assessment using the levels of dioxin-like chemicals found in this study indicate that for the Laysan albatross, ingested plastic particles could explain up to 75% of the body load of dioxin-like chemicals.

TU142

Beyond the Ocean: Plastic particles in limnetic ecosystems

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Plastic waste is of increasing concern in marine ecosystems. Buoyant plastic particles accumulate in pelagic habitats whereas non-floating debris accumulates on the seafloor and in beach sediments, posing risk to the respective communities. Microplastic particles are either directly introduced via sewage discharge or formed by biofouling and mechanical abrasion, making them more prone to consumption by aquatic organisms. As a consequence they can accumulate in higher trophic levels. A variety of harmful effects of plastic and associated chemicals has been shown. However, a large portion of the plastic waste is produced onshore and then enters the marine environment via water discharge to the river systems. Nevertheless, there is a considerable gap of knowledge about the contamination of freshwater ecosystems with plastic particles. We anticipate, that freshwater ecosystems not only act as a source of plastic particles for the oceans, they may also act, at least temporarily, as a sink. This may come along with all the associated harmful consequences which have been reported previously for marine ecosystems. Here we report the occurrence of plastic microparticles in the beach sediment of a subalpine lake, using separation by high density fluids and subsequent identification by Raman microspectroscopy. We show that the amount of macro- and even microplastic particles is reaching similar magnitudes as found in marine environments, suggesting that freshwater systems do not only act as a source for marine contamination. Signs of recent break-up into microplastic particles lead to the assumption, that most of the microplastic particles have not entered the lake as small particles but rather were degraded into smaller particles in the lake environment itself. We also demonstrate the unspecific uptake of microplastic in a wide range of freshwater invertebrates indicating the risk of bioaccumulation. The mere existence of microplastic particles in a subalpine headwater suggests an even higher relevance of plastic particles in low-land waters while the ecological and economic consequences of this contamination are far from being understood.

TU143

Detection and analysis of plastics in the watercycle

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Plastics are everywhere and world plastics production has increased from 1.7 Mtonnes in the 1950s to over 280Mtonnes in present times (around 9% per year). The volume is still growing, despite the current global economic crisis. Large quantities of plastic materials end up in the environment after use, while plastic materials are highly persistent. Due to that nature, plastics fragments became especially visible in marine waters due to local gyres creating large patches or the so-called 'plastic soup'. This phenomenon has created public awareness in Europe

and North America. Later, it has been shown that plastic fragments physically affect sea life, such as mammals and birds. While a lot of research efforts have been directed towards the sources and emission data of plastic fragments within the micrometer range and larger, our research is focused on smaller fragments (nanometer to micrometer scale). While the effects of smaller fragments are yet unknown, some studies suggests that particles may be taken up and distributed in the human body. Our research is linked to the questions from the drinking water utilities to see whether plastics are present in the sources for drinking water. As plastics are present in surface waters and the outflow of sewage treatment plants, the fate of these particles in drinking water treatment processes is also important. We therefore started work on separation, detection and identification of small plastic materials in the watercycle. First, to establish a measurement technique for plastic detection in the nano to micro range in water sources, experiments on concentration of small fragments have been performed using pre-mixed polystyrene in drinking water. Second, by means of FFF linked to a multi angle light scattering detector it was possible to separate and analyse the different particles. Eventually, the concentration techniques in our research will not only be linked to particle separation techniques but also techniques to identify different polymer types, such as nylon, polyethylene, polypropylene or polyvinyl chloride.

TU144

Embryotoxicity of microplastic particles to the sea urchin Paracentrotus lividus

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Evidence for the presence of microplastics in the marine enviroment and their (possible) effects on marine life has significantly increased over the last few years. To date, however, little or no information is available on the potential effects of microplastics on echinodermata. Here, we studied the effect of microplastics on the larval development of sea urchin (*Paracentrotus lividus*) by exposing them to a combination of fluorescent polystyrene (6 micrometer) and polyethylene (25 micrometer) microplastics. The first results on the embryotoxicity to sea urchin after 48 hrs (post fertilisation) of exposure at different concentrations of microplastics are presented. In addition, we analysed the presence of microplastics in field collected sea urchin from the Western Mediterranean Sea. The results will be discussed in relation to the monitoring and assessment requirements (Descriptor 10 on marine litter) under the European Union Marine Strategy Framework Directive(MSFD).

TU145

Leaching of Plastic Additives to Marine Organisms

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It is often assumed that ingestion of microplastics by aquatic species leads to increased exposure to plastic additives. However, experimental data or model based evidence is lacking. Here we evaluate the likeliness of leaching of nonylphenol (NP) and bisphenol A (BPA) in the intestinal tracts of two species, A. marina (lugworm) and Gadus morhua (North Sea cod) using literature data and a prognostic model analysis. We provide a plastic-inclusive bioaccumulation model that allows calculations of the relative contribution of plastic ingestion to total exposure of aquatic species to chemicals residing in the ingested plastic. The model is parameterised using literature data and uncertainty in the most crucial parameters was accounted for by Monte Carlo probabilistic modelling. Modeled NP and BPA concentrations due to plastic ingestion are compared to present global environmental concentration data. For the lugworm, plastic ingestion may yield NP and BPA concentrations that reach the lower ends of global NP and BPA concentration ranges, and thus in some cases may constitute a relevant exposure pathway. For cod, plastic ingestion yields concentrations that are a factor 1000 (BPA) or 10E6 (NP) lower than the lower ends of global NP and BPA concentration ranges. We conclude that plastic ingestion is irrelevant for direct exposure to species like cod, although exposure of benthivorous fish through consumption of benthic worms may be relevant. The bioaccumulation model is general and can be implemented for a wide variety of chemicals and species. As such, it is a powerful tool for the risk assessment of plastic-facilitated transfer of chemicals to organisms.

TU146

Microplastics contamination in two planktivorous and commercial fish species

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Plastic pollution is a huge environmental concern and affects each marine ecosystem. Plastics are produced by millions of tonnes each year in the world and finally accumulate in oceans. They adsorb many persistent organic pollutants, cause external and internal wounds and provoke blockage of the digestive tract of marine mammals, birds and turtles. Plastics can also threaten marine organisms of small size class in the same way by fragmenting in smaller parts that result in microplastics of less than five millimetres. These microplastics are of the same order of magnitude than plankton and can thus be ingested by filter-feeders, suspension-filters and planktivorous organisms such as fish. Few studies deal with microplastics ingestion by fish and even less by commercial fish species. The herring (*Clupea harengus*) and the sardine (*Sardina pilchardus*) were respectively the third and the eighth most caught fish species in the world in 2009. We focused our research on these two species which are of economic importance. We sampled around thirty individuals of each species in the Channel and in the North Sea in January 2013. The stomach contents were digested by sodium hypochlorite and then analyzed. Microplastics were characterized by size, colour and shape. The results of these analyses will highlight the need for studies about microplastics ingestion by planktivorous species.

TU147

Modeling the Fate of Nano- and Microplastics in freshwater systems

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Riverine transport from land based sources constitutes an important pathway of plastic particles to the marine environment. However, fate and transport models for nano-, micro- and millimetre (NMM) sized particles are lacking. Prognostic fate models can be used to assess retention in freshwaters, to assess exposure to aquatic organisms and to quantify transport to sea. Here, we present a novel model for NMM polymer particles implemented for the river Dommel. The spatially and temporally explicit model accounts for advective transport, homo- and hetero-aggregation, sedimentation-resuspension, polymer degradation and burial. Experimental data on particle behaviour as well as literature data are used to parameterize the model. Model behaviour was tested by varying particle radius from 30 nm to 5 mm, for two polystyrene emission scenarios; a point source scenario and a realistic scenario with a combination of diffuse and WWTP (waste water treatment plant) sources. It appears that particle size, biofilm formation and water turbulence have dramatic effects on the fate and retention of NMM sized polymer particles in the Dommel catchment and on the positioning of the accumulation hot spots along the river.

TU148

Producing fragmented micro- and nano-sized expanded polystyrene particles with an accelerated mechanical abrasion experiment

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Microplastics are world widely found from beach to open ocean and from sea surface to deep-sea bed. They are manufactured as small plastic particles (primary microplastics) to produce resin pellets, scrubbers for cosmetics, or blasting materials or they are generated by the fragmentation of larger plastic products (secondary microplastics). Fragmented secondary microplastic particles account for the majority of microplastics and have various origins, which makes proper control difficult. Photo-oxidation and mechanical abrasion on beaches and (or) sea surface are thought to be major weathering and fragmentation process for generating secondary microplastic particles. None of scientific information is, however, available where and how secondary microplastics are produced. Fragmentation of expanded polystyrene (EPS), one of top three polymer types in marine debris monitoring study was done with an accelerated mechanical abrasion experiment in a laboratory. Forty EPS spherules detached from a EPS float were placed in an amber bottle with glass bead (3 mm in diameter) or natural sand (pre-combusted at 450 °C), respectively. The bottles were rotated with a tumbler for a month at 113 rpm. Fragmented EPS particles were extracted by density separation with deionized water and identified with microscopic FT-IR, SEM and fluorescence microscope after Nile Red staining. After mechanical abrasion, apparent surface damage of EPS spherules was observed by SEM analysis. The hundreds of micron scale EPS particles were identified with FT-IR. The EPS particles were selectively stained with Nile Red and subsequently identified and quantified under a fluorescent microscope. EPS particles obviously outnumbered the control and were quantifiable. Number of EPS particles was 5.15×10^5/bottle. About 85% of the EPS particles were within size range of 1-25 mm in maximum length. SEM with energy dispersive spectroscopy revealed that a number of nano-sized EPS particles were also produced during the mechanical abrasion experiment.

TU149

Variability in spatial distribution of micro-plastics in high-tidal coastal environments

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Microplastics (MPs) contribute as a transporter medium of ab-/adsorbed pollutants to higher trophic organisms through marine food-web and to other regions via current. The MPs observed in marine environments comprises of a number of ingredients, experiencing the different weathering process and rates and thus different sorption/desorption rate. Therefore, the fate and transport of MPs is of a big concern in the restoration of marine environments. By not only active physical mixing by a high-tidal range but also a number of emission sources, spatial variability in the distribution and fate of MPs could be amplified in coastal region. This study aimed to investigate the variability in space and time of MP composition and occurrence in the coastal beach sediments and seawaters. Inner-, middle-, and outer-stations longitudinally from the coastal line were surveyed for surface microlayer water (SML) and subsurface water (SSW), and samples of about 10 stations were collected from several sand beaches with different current/tidal direction. MPs in all of samples were categorized with size (>5 mm, 4-5 mm, 3-4 mm, 2-3 mm, 1-2 mm, 0.5-1 mm, 0.3-0.5 mm, and 0.05-0.5 mm) and ingredients (polypropylene (PP), polyethylene (PE), polystylene (PS), expanded polystylene (EPS), polyvinylchloride (PVC), and dyes). Dominant size and ingredient station were compared among each station of the same beach, different beaches, and different water stations. According to those analyses, the main force driving spatial variability was discussed.

TU150

Partitioning of selected hydrophobic organic chemicals between microplastics and seawater

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The occurrence of microplastics (MPs) in the ocean is an emerging world-wide concern. Although sorption of hydrophobic organic contaminants (HOCs) to microplastics may play an important role in the transport processes of HOCs, quantitative evaluation of sorption capacities of various plastic materials is scarce. Most of earlier studies focused on sorption of HOCs to passive sampling material such as low-density polyethylene. In this study, we measured partition coefficients between MPs and seawater (K_{MPsw}) for 8 polycyclic aromatic hydrocarbons (PAHs), 4 hexachlorocyclohexanes (HCHs) and 2 chlorinated benzenes (CBs). Three different polymer materials (i.e., polyethylene, polypropylene, and polystyrene) were chosen because they are the major components of microplastic debris found in the ocean. A third-phase partitioning method was used for the reliable determination of K_{MPsw} to avoid problems with the limited solubility of HOCs in seawater and long equilibration time. First, partition coefficients between polydimethylsiloxane (PDMS) and seawater (K_{PDMSsw}) weremeasured. For the determination of K_{MPsw}, the distribution of HOCs between PDMS or plastics and solvent mixture (methanol:water = 8:2 (v/v)) was determined after apparent equilibrium up to 12 weeks. Laboratory prepared plastic debris (320-440 μm in the longest dimension) was prepared by physical crushing. Partition coefficients between polyethylene and seawater obtained for model HOCs in general agreed well with experimental partition coefficients between low-density polyethylene and water in the literature. The values of K_{MPsw} were generally in the order of polystyrene, polyethylene, and polypropylene for most of the chemicals tested. The ranges of log K_{MPsw} were 2.04–7.87, 2.18–7.00, and 2.63–7.52 for polyethylene, polypropylene, and polystyrene, respectively. The partition coefficients of plastic debris can be as high as other frequently used partition coefficients, such as 1-octanol.water partition coefficients (K_{ow}) and log K_{MPsw} showed good linear correlations with log K_{ow}. High sorption capacity ofmicroplastics implies the importance of MP-associated transport of HOCs in the marine environment.

Detection and characterisation of nanomaterials in complex aqueous matrices (P)

TU151

COUPLING ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION WITH ORBITRAP HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF (FUNCTIONALISED) AQUEOUS FULLERENE AGGREGATES.

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Because of their chemical and electrical properties, nanoparticles such as fullerenes have been widely applied in personal care products, drug delivery systems and solar cells. The fate of manufactured nanoparticles have been increasingly studied because of their potential risks to the environment and human health. In commercial applications such as organic photovoltaic cells, derivatised fullerenes are used to modify their solubility and electronic properties. Aim of the present work is to develop a method that allows the detection, size determination and quantification of fullerenes (and their clusters) in aqueous matrices by means of FFF connected to a MALS detector and an Orbitrap -MS. This would allow the detection of fullerene clusters at environmentally relevant concentrations. Additionally, due to the MS the fullerenes can be identified. This cannot be achieved by UV or MALS alone. The FFF/MALS-analysis of aqueous samples of fullerenes showed that the sizes of the aggregates range between 4 nm to 200 nm. The fractogram demonstrates that the void peak and the first fullerene clusters are clearly separated. Analysis by FFF coupled to the Orbitrap confirmed that the particles observed are fullerene (clusters). Furthermore, we can analyse samples that contain mixtures of different fullerene derivatives.

TU152

Potential of hyperspectral imaging microscopy for semi-quantitative analysis of nanoparticle uptake into cells

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Imaging and subcellular localization studies of engineered NPs often require fluorescent labeling of the particles or use of intrinsically fluorescent NPs such as quantum dots. While allowing the use of widely available fluorescence imaging techniques the attachment of fluorophores could significantly modify the properties of the NPs and influence their interactions with cells and organisms. To date the most often employed methods for imaging of non-fluorescent NPs are electron microscopy based techniques coupled to X-ray spectroscopy. While electron microscopy imaging has significantly higher resolution than light based microscopes, its disadvantages include expensive instrumentation and laborious sample preparation which could introduce artifacts in the specimens. In recent years, alternative methods for imaging of non-fluorescent NPs have been developed and are increasingly used, including hyperspectral imaging with enhanced darkfield microscopy (HSI-M). HSI-M offers the possibility of localizing different NP types in heterogeneous samples. Additionally, sample preparation is fast and easy, reducing the number of potential artifacts that are much more easily introduced in other nano-related microscopy techniques. In this study HSI-M together with dark field fluorescence imaging was used for the detection and subcellular localization of silver and gold NP at sublethal concentrations in a unicellular model organism (protozoan *Tetrahymena thermophila*). In addition, a mixed exposure to fluorescent quantum dots and silver NPs was performed to evaluate the detection limits and differentiation capabilities of HSI-M. The comparison of the uptake of different types of engineered NPs by the protozoan was done using a semi-quantification approach based on the number of mapped pixels containing NP-specific spectra per cell. Shifts in particle spectral characteristics occurred during exposure due to interaction of NPs with protozoan exudates and were taken into account during analysis. Data obtained with HSI correlated with bright field analysis of agglomerates in the cells, suggesting, that HSI-M could be a suitable technique for NP-specific detection, localization and semi-quantification in cells.

TU153

Characterization and behavior of CdTe quantum dots in the aquatic environment: effects of salinity, pH and natural organic matter

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Quantum dots (QDs) are semiconductor metalloid-crystal structures with a nanometer diameter (2 - 100 nm) that contain a metalloid crystalline core coated with a shell or ligands. A great capacity to recognize specific cellular targets, strong fluorescence at narrow and size-tunable wavelengths, resistance to photobleaching, electronic and catalytic properties, makes QDs one of the most exploited nanoparticles (NPs) not only in nanomedicine but also in pharmacy, biology and electronics. However, these NPs can be released into the marine environment, where their toxicological properties and fate are unclear. Thus, the study of physico-chemical characteristic and behavior of QDs in the aquatic environment is off extreme relevance to understand their fate, bioavailability and effects in aquatic organisms. Accordingly, the main objective of this study was to characterize orange QDs (2-7 nm, CdTe core covered with carboxyl groups) in terms of aggregation kinetics, surface charge, dissolution and complexation under environmentally relevant conditions of salinity, pH and natural organic matter (NOM). QDs (40 $mg.L^{-1}$) were suspended in three aqueous media: ultrapure water (18 MΩ/cm), $NaNO_3$ 100 mM and natural seawater (S = 36.3), over a wide range of pH (1.7 - 12) and analyzed using Dynamic Light Scattering (DLS), Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) and Stripping Chronopotentiometry

(SCP). The results on the absorption spectrum at 530 nm confirmed that the QDs used present the characteristic structure of semiconductor nanocrystals. The DLS analysis showed that the QDs also have a higher sedimentation rate in seawater (0.88 ± 0.0005) than in ultrapure water (0.70 ± 0.0016), associated with a higher aggregation and a zeta potential near to zero (-9.37 ± 1.16 and -42.66 ± 0.55, respectively). Salinity changes the isoelectric point of the QDs in the aqueous environment (1.7 and 2 in ultrapure water and $NaNO_3$ and 10 and 12 in seawater), while the pH and NOM are significant parameters for the aggregation state of the NPs in the exposure media. Furthermore, the AGNES and SCP analyzes suggest the existence of a process of degradation, dissolution and complexation of the QDs in seawater. Overall, this study indicates that the physicochemical properties of NPs and the environmental conditions may affect the bioavailability and fate of NPs in the aquatic environment and consequently their toxicity to aquatic organisms.

TU154

Characterization of silver nanoparticles in aqueous matrices using asymmetrical flow field-flow fractionation

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The development of methods to monitor manufactured nanomaterials in the environment is one of the crucial areas for the assessment of their risk. More specifically, particle size analysis is a key element, because many properties of nanomaterial are size dependent. However the sizing of nanomaterials in the realistic environment is very challenging due to their heterogeneity and reactivity with other environmental components. In this study, the fractionation and characterization of polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNPs) with three different sizes was investigated using asymmetrical flow field-flow fractionation (As-Fl-FFF) coupled with UV-Vis spectrophotometry and Dynamic light scattering (DLS). In particular, effects of ionic strength and natural organic matter (NOM) on the particle size and stability were evaluated. The fractogram peaks of three different AgNPs decreased as the ionic strength increased, while the fractogram peaks increased in the presence of humic acid. In addition, particle size slightly increased in the presence of humic acid, suggesting the adsorption of humic acid onto the surface of AgNPs and resulted in steric stabilization of the particle suspension. It is interesting to note that batch DLS measurements could not separate the three different particles and could no detect the minimal particle size change. These results imply that the application of As-Fl-FFF coupled with highly sensitive detectors could be a powerful method to analyze the behavior of AgNPs at different physicochemical conditions. The described method will also open the door to obtain reliable data on the occurrence and the behavior of other manufactured nanomaterial in the environment.

Fate and effects of nanomaterials in soil (P)

TU155

Effect of silver nanoparticles in the microbiome of the isopod Porcellionides pruinosus

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Widespread use of silver nanoparticles (AgNPs) in daily products leads to their guaranteed presence in the environment; as a result, the knowledge of its toxicity on organisms and associated microbial communities is crucial to relate individual effects with ecological functioning. Disturbances induced by antimicrobial AgNPs and its counterparts may have severe consequences on the composition of the microbiome of isopods, possibly causing impairment of soil function. This study represents the first attempt to explore this hypothesis and is important to recognize the environmental risk associated with NPs. Ecotoxicological bioassays were performed to determine the possible effects of AgNPs in the microbiome of the gut and feces of the terrestrial isopod *Porcellionides pruinosus*. Isopods were exposed to 50mg/Kg (NOEC- no observed effect concentration) and 5μg/Kg (PEC-predicted environmental concentration) of AgNPs and the ionic counterpart ($AgNO_3$). Microbial communities of isopods' gut and feces were analysed by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified fragments and by massive pirosequencing of the 16S rRNA gene. Sequences were compared with sequences available in public databases to determine their phylogenetic closest relatives. Results revealed that exposure to both Ag forms alter the microbiome of *Porcellionides pruinosus*. Although PCR-DGGE analysis detects only ribotypes that represent at least 1% of the total community, it allowed identifying effects of AgNPs and $AgNO_3$ on the microbial community of isopods' gut and feces. By using this methodology, no distinct effects were observed on the microbial community composition when exposing isopods to the two tested concentrations. However, it is evident that AgNPs and $AgNO_3$ have distinct antimicrobial effects, disturbing dissimilar microbial communities of the isopods' gut and their feces. This study indicates that AgNPs impact the isopod's microbiome. Thus, NP contamination of soils, particularly by silver NPs, is a concern and future investigation for establishing a link between microbiota changes and possible impairment of soil function are needed.

TU156

Internalization and depuration of silver nanoparticles in earthworm coelomocytes

J. Kwak; Y. An, Konkuk University / Department of Environmental Sciences

Recently, flow cytometry were used to assess the internalization of nanoparticles in mammalian cells, human epithelial cells, bacteria, and earthworm coelomocytes with analysis of side scatter of light. In the present study, we investigated the internalization as well as depuration potential of PVP coated silver nanoparticles (pAgNPs) in earthworm coelomoctytes using flow cytometric analysis via *in vitro* assay. Also inhibition of esterase activity was investigated using flow cytometer after calcein-AM staining. As results of in vitro assay, significant inhibition of esterase activity was observed at 10 mg/L of 50 nm pAgNPs, 20 mg/L of 100nm and 150 nm pAgNPs after one hour exposure. With flow cytometric analysis of SSC, rapid internalization and depuration of pAgNPs in earthworm coelomocytes were measured. Although nanoparticles were depurated rapidly, residues of nanoparticles in the cells were increased with incresing exposure concentrations.

This study was supported by the research project for Environmental Risk Assessment of Manufactured Nanomaterials (KK-1303-03) funded by the Korea Institute of Toxicology (KIT, Korea).

TU157

Effects of TiO2 nanoparticles on the symbiosis of red clover and Rhizobium trifolii in a hydroponic system

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In future, nanoparticles (NPs) might be applied on agricultural fields, as their use in plant protection is discussed in patents and publications and prominently involving NPs based on TiO_2. Application of TiO_2 NPs in agriculture could affect crops, soil microorganisms as well as their interactions. Clover is an important fodder crop and serves as green manure due to the symbiosis with rhizobia. To better understand how TiO_2 NPs could interact with crops and soil microorganisms, a small scale hydroponic system was developed using red clover and *R. trifolii* as model organisms. Two concentrations of two different TiO_2 NPs, i.e., P25 and E171, were used to assess effects on plant growth, nodule formation and N_2-fixation. TiO_2 NPs (P25 and E171) as well as bulk TiO_2 were suspended (24 mg l^{-1}) in Fåhraeus medium and $ZnSO_4$ (0.016 g l^{-1}) was used as positive control. Red clover seedlings were transferred to the small scale hydroponic system in 20 ml glass tubes containing the different media and *R. trifolii*. NP size (nanoparticle tracking analysis) and concentration (ammonium persulfate digestion and inductively coupled plasma optical emission spectrometry) were measured during the four week exposure. At harvest, main root length, shoot length, dry weight and number of nodules were determined. Both TiO_2 NPs (E171 and P25), bulk TiO_2 at 24 mg l^{-1} and the positive control $ZnSO_4$ decreased shoot dry weight (38-46 %) and root dry weight (32-39 %). Reduction of the length of the main root (28-35 %) and the shoot (41-65 %) was detected in all of the treatments. While all of six replications of the control plants formed nodules, 33% P25, 33% E171 and 50 % bulk TiO_2 treated plants did not nodulate. Clover growth as well as the symbiosis with rhizobia was negatively affected by TiO_2 NPs in a hydroponic model system. Attachment of the TiO_2 NPs onto the root surface were observed and could affect nutrient uptake, root growth and nodule formation. Further experiments will show whether red clover, *R. trifolii* and their symbiosis are also affected in soil on a larger time scale.

TU158

Behavior of copper oxide nanoparticles in soil and their totxicity to Paronychiurus kimi (Collembola)

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The metal oxide nanoparticles which have been used widely have potential toxicity due to its high surface area and unstable physicochemical properties. In this study, toxic effects of the copper oxide nanoparticles (CuO-NP) to *Paronychiurus kimi* were evaluated in soil system which has been studied fewer compared with aqueous phase. To investigate the toxic effect, the tests which were to determine the behavior of CuO-NP were also conducted. First, the adsorption capacity of CuO-NP was estimated with Freundlich sorption constants (K_d) and the amount of copper ion and nanoparticles in pore-water were measured. Second, the toxicities of CuO-NP and CuO-NP with dispersant agent (PAH, PAA) to *P. kimi* were assessed in accordance with ISO 11267. After 28 day exposure, LC50 for survival adult and EC50 for reproduction were estimated. To assess biochemical effect, antioxidant enzyme activity of survival adults was measured and protein was profiled using SELDI-TOF MS. Most amounts of CuO-NP and non-nano CuO were adsorbed to

soil particle and few copper ions were released to pore-water. PAH and PAA used as dispersant of CuO-NP were ineffective in soil as compared with aqueous base. The result of toxicity on Collembola was not significant for their reproduction and antioxidant enzyme activities. However, protein profiling result showed that the protein pattern of the organisms exposed to CuO-NP was similar to that of $CuCl_2$ treatment which have same copper ion concentration with released from CuO-NP. Based on the results, the cause of the effect on *P. kimi* should be copper ion not related to nanoparticles itself.

TU159

The toxicity of phenanthrene to the soil organisms Porcellionides pruinosus and Folsomia candida in the presence of CeO2 nanoparticles

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The use of cerium oxide nanoparticles (CeO_2 NPs) as fuel additive in diesel is a promising improvement in emission reductions. In spite of the benefits of using CeO_2 NPs, there may be risks of combined exposure to CeO_2 and polycyclic aromatic hydrocarbons in the environment, as they are likely to co-exist. Phenanthrene is a major component of diesel exhaust particles and therefore a common pollutant in the environment. This research aims at evaluating the influence of CeO_2 NPs on the toxicity of phenanthrene to two soil organisms, the isopod *Porcellionides pruinosus* and the springtail *Folsomia candida*. Toxicity tests were performed using three CeO_2 NPs concentrations up to 1000 mg Ce/kg dry soil and six phenanthrene concentrations up to 480mg/kg for *P. pruinosus* and up to 160 mg/kg for *F. candida*. A feeding inhibition test (14 days) and a reproduction test (28 days) were conducted in Lufa 2.2 soil with isopods and springtails, respectively. LC50 and EC50 values were calculated using a logistic model and compared for significant differences between CeO_2 NPs concentrations using a generalized likelihood ratio test. No mortality was observed in isopods exposed to CeO_2 NPs up to 1000 mg Ce /kg dry soil. However, in all treatments with and without CeO_2, phenanthrene showed a dose-related effect on isopod survival, with LC50s of 110-143 mg/kg regardless of the CeO_2 NPs concentration. Isopod biomass also dose-related decreased with increasing phenanthene concentration in soil, with EC50s of 17.6-31.6 mg/kg regardless of the CeO_2 NPs concentration. The LC/EC50 values for the toxicity of phenanthrene to the isopods were not significantly affected by CeO_2 ($X^2_{(1)} < 3.84$). For the springtails, survival and reproduction were reduced in a dose dependent manner by phenanthrene. LC50s ranged from 65 to 88 mg/kg, EC50s from 52 to 77 mg/kg and EC10s from 25 to 70 mg/kg. No effect of the CeO_2 NPs on springtail survival and reproduction was observed, and the different CeO_2 NPs levels did not influence the toxicity of phenanthrene. This study is the very first report on phenanthrene toxicity to the isopod *P. pruinosus*. Toxicity of phenanthrene to *F. candida* was comparable to the toxicity data reported before. In conclusion, generally no effect of CeO_2 NPs on phenanthrene toxicity to isopods and springtails was observed.

TU160

Toxicity of silver and zinc oxide nanoparticles to the terrestrial isopod Porcellionides pruinosus

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The expanding range of applications for nanoparticles (NPs) led to the release on the market of hundreds of NP-containing products. Due to their widespread use, NPs are likely to enter into the environment, with soils being an important final sink. Silver and zinc oxide nanoparticles (Ag-NPs and ZnO-NPs, respectively) are among the most commonly used NPs present in daily care products mainly due to their antibacterial properties. Some studies have demonstrated that the input of Ag-NP and ZnO-NP into the environment may cause adverse effects on ecoreceptors, implying that these NPs are emerging contaminants of concern. The aim of the present work was to evaluate the toxic effects of Ag-NPs and ZnO-NPs and their respective ionic forms (Ag^+ and Zn^{2+}) on the terrestrial isopod *Porcellionides pruinosus* using different soil types. For this, feeding inhibition tests and avoidance behaviour assays were performed. Soils from Portugal, The Netherlands, and the standard Lufa 2.2 soil from Germany were used in order to investigate the influence of soil properties on the toxicity. In general, higher toxicities were found for the ionic forms rather than the NP forms. Silver (NP and ionic forms) showed higher toxicity on isopods feeding performance than zinc. Lower EC_{50} values for food consumption ratio were found in the isopods exposed to the soil from Portugal. The results from avoidance behaviour tests indicated that isopods can detect and avoid NPs at very low concentrations. The present study showed that soil properties are very important in determining the toxicity and bioavailability of NPs in soils and should be also taken into account under risk assessment.

TU161

Soil type influence on Ag Nanoparticles by earthworms, Eisenia fetida

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Earthworms are key sentinel organisms playing an important role in improving the soil structure. Here we tested the importance of soil type on the toxicity to silver nanoparticles (Ag NPs) to earthworms, *Eisenia fetida*. Silver nanoparticles are widely used in a range of consumer products mainly as antibacterial agents and thus causes potential risk to the environment once these particles are released into the environment [1]. In our tests, we were able to show that the earthworm toxicity was strongly dependent on the soil type, with strongest effect in low organic matter soil. Studies on the organic matter content, clay and cation exchange capacity along with the metal solution activity will give insight into the bioavailability of metals in different soils; hence for each of the soil type the fate of the AgNPs was also measured.

TU162

Fate of silver nanoparticles after uptake into earthworms tissues – dissolution and/or excretion?

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The use of silver nanoparticles (Ag-NPs) has increased in the last years, with soils being one of the main environmental sink after their use and release. Various short term tests of metal NPs effects on different terrestrial organisms are used to determinate their risk in soils. Earthworms are one of the most representative test organisms for soil effect assessments. They are exposed to contaminants in soil both through their skin and by soil ingestion (gut). Previous studies have demonstrated that earthworms take up the majority of their Ag through the gut, and when exposed to Ag-NP, they build up excessive Ag internal concentrations that cause little effect compared to known effect thresholds for Ag ions. Thus, it is important to investigate the mechanisms by which the different Ag species enter, deposit in and are eliminated from the body of the exposed organisms. The aim of this study is to estimate whether the accumulated Ag-NP is eliminated over time or otherwise changed into a toxic form into cells and tissues where Ag-NPs may be stored. For this experiment, a natural soil (LUFA 2.2) was spiked with $AgNO_3$ and Polyvinylpyrrolidone (PVP) coated Ag-NP. A low effect Ag concentration of 141 mg Ag kg^{-1} d.w., was selected from previous toxicity data. In each treatment, ten adult *Eisenia fetida* earthworms, with fully developed clitellium, were exposed to the spiked soils for 28 days at 20º in a climate chamber. At day 28, earthworms were removed and the soils from this first set (set 1) retained for reproduction assessment. Some worms were transferred to clean soil to start an elimination phase (set 2) and the rest transferred to clean soil to follow possible reproduction recovery (set 3). Ten earthworms were removed from set 2 at 0, 4, 8, 24, 36, 48, 72, 96, 120 and 168 hours during the elimination phase and depurated for 24 hours before freezing. To test recovery in reproduction, earthworms from set 3 were transferred to fresh clean soil (set 4) after 2 weeks. To assess effect on reproduction the number of juveniles from set 1, 3 and 4 were counted after 28 days of further incubation. By measuring the total internal silver concentrations at the different sampling times we will assess whether the worms have the ability to eliminate silver from their gut. Furthermore, we will be able to assess if the over accumulation of Ag from the NP exposures will be have longer term effects on reproduction.

TU163

In vivo cytotoxicity of silver nanoparticles in three different sizes with earthworms

J. Kwak; Y. An, Konkuk University / Department of Environmental Sciences

This study investigated acute toxicity and *in vivo* cytotoxicity of silver nanoparticles in three different sizes using earthworm. *Eisenia andrei* were selected as test species and exposed to 50 nm, 100 nm, and 150 nm PVP coated silver nanoparticles (pAgNPs) in the OECD artificial soil for 14 days. ROS production and intracellular esterase activity were assessed by flow cytometer after staining of $DCFH_2$-DA and calcein-AM, respectively. Effects of dissolved silver ion and PVP coating agent were also evaluated. Acute toxicity and cytotoxicity of pAgNPs were observed at the concentration of 2000 mg/kg dry soil. Negligible adverse effects of pAgNPs on earthworm survival and abnormalities were observed. pAgNPs with smaller size did not show greater cytotoxicity in terms of ROS production and interacellular esterase activity.

This study was supported by the research project for Environmental Risk Assessment of Manufactured Nanomaterials (KK-1303-03) funded by the Korea Institute of Toxicology (KIT, Korea).

TU164

Induced genotoxicity by silicon nanoparticles with different Zeta potential on earthworm coelomocytes

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We tested the use of surface Zeta potential (Z-P) measurements as a tool to investigate the interactions of Si nanoparticles and their genotoxicity with

celomocytes extruded from earthworms. Our experimental system was made with SiNP using as shell a bi-layer coating of poly(allylamine hydrochloride) (PAH) and poly (sodium-4-styrene sulfonate) (PSS) and as fluorophore, Fluorescein isothiocyanate (FITC) to obtain finally SiNP-PAH FITC-PSS. We work with two types of NP: 250nm, Z-P: +54mV and 253.3nm, Z-P: -46mV. Size measured by Dynamic Light scattering. Cells were incubated respectively with two types of nanoparticles, the significant differences in their surface charge change indicate the potential role of Zeta potential as a valuable biological signature in studying the cellular interaction of nanoparticles, as well as specific cell genotoxicity.

TU165

Single and mixture toxicity of gold nanoparticles and gold(III) to Enchytraeus buchholzi (Oligochaeta)

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The ecotoxicity of gold nanoparticles (Au-NPs), gold(III) and their mixtures was assessed in the potworm *Enchytraeus buchholzi*after 14-day bioassays. The worms were exposed at 20°C in OECD artificial soil to 0, 9.375, 18.75, 28.125 and 37.5 mg/kg Au-NPs or Au(III) and 0, 9.375 + *9.375*, 18.75 + *18.75*, 28.125 + *28.125*, 37.37 + *37.5* mg Au-NPs + *Au(III)*/kg. The results indicated that the range of Au-NPs concentrations used in the present study was not deleterious to the survival and reproduction of *E. buchholzi*. Au(III) showed no significant effect on survival but was statistically deleterious to reproduction in the highest treatment ($p \leq 0.01$). A 14-day $EC50_{Au(III)}$ = 35.506 mg/kg was estimated for reproduction. Au-NPs/Au(III) mixtures essentially caused the same effects as Au(III) except for 18.75 mg Au(III)/kg vs. 18.75 + *18.75* mg Au-NPs + *Au(III)*/kg where the mixture treatment was significantly more toxic to reproduction ($p < 0.02$). Mixture results suggested a less than additive effect between Au-NPs and Au(III), with Au(III) being the probable major contributor to the observed toxicity. The order of toxicity between Au-NPs, Au(III) and their mixtures was Au-NPs = Au(III) = Au-NPs/Au(III) for survival and Au-NPs < Au(III) ≤ Au-NPs/Au(III) for reproduction.

TU166

Effect of trophic transfer of quantum dots in soil food chain

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The soil food chain is very complicate and difficult to verify the nanoparticles (NPs) trophic transfer. Trophic transfer and biomagnification of NPs may be significan process, and the adverse effects and their mechanisms should be obviously demonstrated. In this study, we consisted the simple soil food chain, and evaluated the effects of NPs trophic transfer. Quantum dots were chosen as test NPs, and the *Escherichia coli* (strain OP50) and *Caenorhabditis elegans* were selected as test species. *E. coli* was exposed to QDs-LB media, and then *C. elegans* fed the *E. coli* on nematode growth media (NGM). The general endpoints such as cell growth, survival, and reproduction were observed. To assess the QDs uptake and its oxidative stress, flow cytometry and fluorescence microscopic analysis were employed using calcein acetoxymethyl ester (Calcein AM) and DCFH (Dichloro-dihydro-fluorescein) as fluorescent dyes for indicators of esterase activity and oxidative stress. It was observed that survival, growth, and reproduction of *E. coli* showed no significant difference compared to the control group. However, the DCFH intensities of *E. coli* were increased when they were exposed to QDs, indicating that ROS were enhanced by QDs on each trophic levels, and it can be related with oxidative stress. These results may be primary evidence of effect of NPs trophic transfer in soil food chain.

Usage, fate and risk of carbon based nanomaterials (P)

TU167

Increased sensitivity of second generation Hyalella azteca to the nanocomposite Carbo-Iron

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In the project Fe-NANOSIT, Carbo-Iron®, a composite of activated carbon and nano Fe^0, is currently used in a pilot study for remediation of halogenated hydrocarbons in a contaminated aquifer. Potential ecotoxicological effects of Carbo-Iron in the aquatic environment have been investigated with standard tests. As beyond the contaminated zone Fe^0 is oxidized to Fe^{2+}/Fe^{3+}, oxidized Carbo-Iron is used in these tests. During remediation and in ecotoxicological tests, Carbo-Iron is stabilized with carboxymethyl cellulose. Higher crustaceans are important groundwater organisms and were shown to be more sensitive to some contaminants than lower crustaceans. Therefore, an acute and a reproduction test with the amphipode *Hyalella azteca* were performed based on US EPA 600/R-99/064 (2000). The investigated Carbo-Iron concentrations were 6.3, 12.5, 25, 50 and 100 mg/L, additionally a control and a dispersant control were included in each experiment. Particle size was determined in stock and test suspensions via dynamic light scattering and uptake of particles into test organisms was investigated with microscope based methods. In a follow-up study, the reproduction test was modified: 30 d old precopula pairs of *H. azteca* were exposed to oxidized Carbo-Iron for 56 d, offspring was collected 3 times during the test from each treatment and allocated to 10-d acute toxicity tests. Particle size in the suspensions ranged from 258 to 408 nm. Acute effects of oxidized Carbo-Iron on biomass of *H. azteca* were detected at 100 mg/L, while 50 and 100 mg/L significantly reduced the number of offspring in the reproduction study according to EPA 600/R-99/064. Carbo-Iron deposits were detected on the carapax and in the gut of *H. azteca*, but no transfer into cells of the surrounding tissue was observed with the methods applied. In the modified reproduction test, survival of adults and the cumulative number of offspring were reduced at concentrations ≥ 25 mg/L and ≥ 12.5 mg/L, respectively. Acute tests with offspring collected during exposure revealed an increased sensitivity of organisms originating from the treatments as compared to previously unexposed organisms.

TU168

Effect of the NM presence on the bioavailability of flame retardants

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Due to the increased use of nanomaterials (NMs) nowadays, more and more studies are being carried out to know their environmental behaviour. Some of these studies suggest that NMs can absorb hydrophobic contaminants on its surface. The objective of our study is to know if the presence of these NMs in rivers could affect the available fraction from sediments of other contaminants, such as flame retardants (FRs). This available fraction is the one that can be actually be absorbed by the fish living in the river, which is more important than the total concentration of contaminant in the sediment. We selected a number of eight polybrominated diphenyl ethers (PBDEs), three novel brominated flame retardants (BFRs) and five halogenated norbornenes (HNs) to test the effect of the presence of NMs. First of all, we determine the bioavailability of selected FRs using Tenax® extractions. Tenax is a polymer that absorbs on its surface the hydrophobic contaminants. Two grams of spiked sediment were put in a separator funnel with 40 mL of water and 2 grams of Tenax. The Tenax was refreshed eight times up to completing a period of 432 h. Then the Tenax was analyzed to know the amount of FRs desorbed from the sediment. Results obtained during the different times allowed to determine the desorption kinetics of each contaminant. Bioavailability of FRs ranged from 35 to 79% for PBDEs, from 32 to 66% for novel BFRs, and from 35 to 70% for HNs. Then, the same sediment was also spiked with C_{60} and C_{70} in a concentration fifty times higher than those found in the environment (50 ng/g dw). Desorption kinetics were repeated with the presence of NMs, and results were compared with and without NMs. We did not see any significant difference on the FR bioavailabilities between both experiments. Finally, we repeated the tests with presence of NMs, but increasing the concentration level present in the sediment (25000 and 100000 ng/g dw). In this case, and with high amount of NMs, results showed that bioavailability of FRs decreased with the presence of fullerenes, except for three compounds: DBDPE, Dec-602 and DPMA. The decrease was between 20% (for BDE-99) and 87% (for BDE-209). This behaviour could be explained by the absorption of these contaminants on the surface of the NMs present in the sediment. **Session:** Fate and effects of nanomaterials **Keywords:** Bioavailability,Flame retardants, Nanomaterials, Tenax extraction **Presentation preference:** Poster

TU169

Toxicity of unmodified and functionalized diet-borne C60 fullerene to the freshwater snail Lymnaea stagnalis

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The toxicity of diet-borne fullerenes was evaluated by exposing freshwater snails *Lymnaea stagnalis* to spiked agar pellets containing tetraphyll as nutritional source. Snails were exposed for 4 weeks to unmodified C_{60} in three different concentrations, i.e. 0, 30, and 100 mg/l agar. At the end of the exposure period, snails were dissected, and analyzed for oxidative stress markers, cellular energy allocation, and histopathological effects. No statistically significant effects were found on growth and reproduction, although results suggested a disturbed (less stable) egg laying pattern and a decrease in lipid stores in exposed snails. No indications were found for increased oxidative stress in digestive glands, nor for any histological damage in reproductive tissues. In a follow-up experiment, snails were exposed for 4 weeks to a single 100 mg/l agar concentration of either C_{60} or three of its functionalized derivatives, i.e. [6,6]-phenyl C_{61} butyric acid methyl ester, [6,6]-thienyl C_{61} butyric acid methyl ester, and N-methylfulleropyrrolidine. Growth and reproduction were again not affected by the exposures, but a significant decrease was observed in superoxide dismutase (SOD) activity in digestive glands from snails exposed to the functionalized fullerenes, but not to the unmodified C_{60}.

Other oxidative stress markers still have to be analyzed. Results so far indicate that exposure to relative high concentrations of food-borne fullerenes seems to be a minor risk for *Lymnaea stagnalis*. By an analytical characterization of C_{60} and its functionalized derivatives in the food (agar) and in the snail tissues, we are currently investigating if this low risk can be attributed to the nature and the extent of exposure.

TU170

Green algae interacting with single-walled carbon nanotubes affect the feeding behaviour of mussels, mitigating nanotube toxicity

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Abstract The increased industrial application of carbon nanotubes has led to a significant interest in their aquatic ecotoxicology and potential for trophic transfer. Green algae (*Tetraselmis suecica*) were exposed in triplicate to 5µg L^{-1}, 10µg L^{-1}, 50µg L^{-1}, 100µg L^{-1} and 500µg L^{-1} single-walled carbon nanotubes (SWCNTs) for 8 days. Light microscopical observations, confirmed by SEM and Raman spectroscopy, showed that SWCNTs adhered to the external algal cell walls. TEM results suggested SWCNTs may have been internalized by the algae. A direct effect of SWCNT exposure on the algae was a significant ($P< 0.001$) decrease in chlorophyll *a* concentrations, accompanied by a significant decrease ($P< 0.001$) in cell viability by day 7 at concentrations of 500µg L^{-1}. We have previously shown that mussels can remove SWCNTs from the water column and reject them as pseudofaeces. In order to study the feeding behaviour of mussels (*Mytilus edulis*) presented with algae in the presence of SWCNTs, known algal concentrations and SWCNT 500µg L^{-1} alone and combined were applied to the tanks and mussels left to feed for 10 minutes, after which mussels were transferred to clean seawater and left to depurate for 24h. The control treatment contained algae without mussels, in order to correct for algal cell division. Selective bivalve feeding was observed using a newly developed flow cytometry technique with pseudofaeces as a proxy. Psuedofaeces production increased significantly ($P=0.008$) under combined algae and SWCNT exposure. DNA damage and oxidative stress were used as ecotoxicological biomarkers of exposure in mussels. A 24h exposure to 500µg L^{-1} SWCNTs showed significantly increased DNA strand breaks in both gill cells and haemocytes ($P< 0.001$), and significantly increased oxidative stress, expressed as superoxide dismutase (SOD) activity ($P< 0.001$) and lipid peroxidation in gills ($P=0.032$). However, when SWCNTs were presented together with algae, DNA damage in haemocytes and gills ($P=0.534$; $P=0.998$) or oxidaitve stress were not significantly increased above control levels ($P=0.981$; $P=0.999$). Mussels appeared to largely reject algae containing SWCNTs. However, SWCNT injested can not be rules out and we are currently investigating whether the observed SWCNT-algal interaction may facilitate trophic transfer of SWCNTs up the food chain with potential consequences for human health.

TU171

Toxicity evaluation in Xenopus laevis tadpoles exposed to multi walled carbon nanotubes under normalized conditions

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Due to their potential in numerous industrial applications because of their exceptional properties, some of carbon nanotubes are expected to get into the environment, and to be found into the aquatic compartment. Nevertheless, ecotoxicological data are still scarce, especially on aquatic organisms. The aim of this study was to evaluate potential adverse effects of industrial Multi-Walled NanoTubes (MWNT, 0.1 to 50 mg/L) in amphibian larvae (*Xenopus laevis*). Several toxicity endpoints were carried out: mortality (12 d), growth inhibition (12 d), micronuclei induction (12 d) and primary DNA damage (comet induction, 2, 4, 8, 24 h, and 12 d) in erythrocytes, and oxidative stress induction in entire larvae (reactive oxygen species production, catalase CAT, superoxide dismutase SOD and glutathione reductase GR after 2, 4, 8, 12 and 24 h of exposure). The general aspect of the exposed larvae was observed. After 12 days of exposure, results show no mortality and no significant micronucleus/comet induction whatever the concentration, but a significant growth inhibition of larvae exposed to 50 mg/L. Results show a high H_2O_2 production depending to the exposure time. Time-related fluctuation was observed with significant peaks of activation: 8 h to 0.1 mg/L, 4 and 8h to 1 mg/L and 2h to 10 mg/L (GR); 4 and 8h to 1 mg/L and 2, 8 and 24h to 10 mg/L (CAT); 2, 12 and 24h to 10 mg/L (SOD). Primary DNA damages are significant after 4 and 24h to 0.1 mg/L and after each time of exposure to 1 mg/L suggesting repair/compensation process in larvae after 12 days of exposure. Presence of MWNTs can be suspected in larvae thanks to their agglomeration in basket gills and intestine from 2 h of exposure. The Raman analysis confirms their presence into the lumen of gut larvae after 12 days but neither in intestinal tissues and cells, nor in the circulating blood of exposed larvae. These data strongly support the fact that the chronic toxicity observed in larvae exposed to high concentrations of MWNTs could be limited to physical effects (gill clogging and/or abrasive effects and/or nutrients deprivation). This mechanic toxicity may lead to oxidative stress as demonstrated by results of oxidative stress (H_2O_2 and enzymes) and primary DNA damage. Induction of the oxidative stress is earlier to the hight concentration of MWCNTs. Profiles of DNA damage, H_2O_2 production and enzymatic activities in the course of time show fluctuations which are characteristics of oxidative stress response.

TU172

Genotoxicologic and histopathologic studies on Zebrafish (Danio rerio) exposed to cup-stacked carbon nanotubes

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Investigation of the fate and behavior of nanomaterials in the environment raises more concerns than true answers. Physicochemical properties, aggregation, solubility and sedimentation of nanomaterials in water might be characterized before and after experiments. Carbon nanotubes (CNTs) aggregate in water, making it very difficult to evaluate a real exposure-concentration in aquatic bioassays. Furthermore, CNTs adsorbed onto water will sediment to the bottom of aquariums being less available to exposed organisms. The potential of CNTs to cause toxic effects to fish species remain controversial because studies have demonstrated that such toxicities more likely resulted from dispersants and surfactants. Lesions observed in gills of zebrafish and rainbow trout exposed to CNTs could also be attributed to physical irritation. The genotoxicity of nanomaterials reported by many authors are related as a result of oxidative stress and are not due to a direct interaction with DNA or chromosome. In our study we evaluated the genotoxic effects of CNTs (cup-stacked) by the comet assay (CA), micronucleus test (MN) and DNA fragmentation by flow cytometry (FC) in peripheral erythrocytes of Zebrafish, followed by histopathological analysis of gills, liver and gut after exposures at 5.0, 10.5 and 22.05 $mg.L^{-1}$ for 96 h. Our results indicated that CNTs did not show toxicity or genotoxicity to zebrafish. After acute exposure at 22.05 $mg.L^{-1}$for 96 h to CNTs, a recovery study was performed for 24, 72 and 144 h post-exposure in clean water. A complete clearance of CNTs from the fish'body was observed after 144 h post-exposure. Presence of CNTs in gut lumen as black masses was confirmed by histologic and Raman spectroscopy analysis. CNTs were not found within epithelial cells of the gut nor in the liver and gills cells. Some inflammatory focus was observed in the gills at exposures of 10.5 and 22.05 $mg.L^{-1}$. Probably, CNTs were adsorbed by gut mucus, acting as a barrier against its penetration into the fish'body. Research supported by CNPq, FAPESP, University of Brasília and University of São Paulo

TU173

Development of an analytical method for the determination of fullerenes in biological matrices

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An analytical approach was set-up for the analysis of fullerenes (e.g. C60) in biological tissues. The main aim was to develop an analytical method to localize the nano-particles (NPs) in biological tissues and to provide information on the spatial distribution of NP in organisms. The approach is based on the development of a screening method for the detection and quantification of NP agglomerates/aggregates in biological tissues followed by a confirmation step. The method was used to detect fullerenes in organisms that were exposed to these particles during toxicity studies in the NanoNext.NL project. As a first step, a comparison and validation of analytical methods was made for the detection and quantification of fullerenes in biota. Liquid chromatography (LC) combined with high resolution Time-of-Flight (ToF) mass spectrometry was optimized for the detection of fullerenes using different ionization techniques (ESI, APCI, APPI). For the validation of the method snails (*Lymnaea stagnalis*) were spiked with fullerenes and different extraction and clean-up methods were evaluated. The recovery studies showed that strong ion suppression with LC-ToFMS occurred for many method extraction methods. Correction for ion suppression with internal standards was necessary to improved the recoveries.

TU174

Ecotoxicological effects of single walled carbon nanotubes against benthic organisms

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The economical crisis experienced by the canadian forestry industry has led to the search of new forestry products. Elaboration of nanomaterials including nanotubes

and single-walled carbon (SWNT) based on those product has been proposed. These possess the most notable features among carbon materials and are closely studied for an increasing number of important applications, including water treatment and drug targeting. Furthermore the addition of functional groups to SWNTs or their association with polymers (functionalization) is more and more investigated, because it allows them to become soluble in water and facilitates their incorporation into various materials. Consequently the probability of finding these different forms of SWNT in the environment is doomed to increase, but there is little data on their ecotoxicological potential especially in aquatic invertebrates. In addition, applications of nanomaterials are growing and some studies have revealed the toxic effects of some of them, mostly because of their unique physical and chemical characteristics. The aim of our study is to identify the ecotoxicological effects of as-produced and purified SWNTs on various benthic organisms. Because nanomaterials are poorly soluble in water they tend to settle down and therefore be directly in contact with those kinds of model organisms. Results of the toxicity tests of as-produced and purified SWNTs will be presented.

TU175

Bioaccumulation and Ecotoxicity of Carbon Nanotubes

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A review of the existing literature on ecotoxicity of CNT has been performed and the results are presented here. Several studies provide evidence that CNT do not cross biological barriers readily. When ingested by living organisms, CNT are subsequently excreted. When internalized, only a minimal fraction translocates into other body compartments. Thus bioaccumulation is limited; however organisms containing CNT may become source of entry of CNT into the food chain, potentially leading to biomagnification. Toxicity depends on exposure, model organism, CNT type and dispersion state. Aquatic organisms are more affected than terrestrial organisms. Invertebrates are more sensitive than vertebrates, with single-walled CNT being more toxic than multi-walled CNT. CNT length and dispersion degree play a role for the toxic outcome. It can be assumed that the ratio length/diameter also plays a role. Hence, the fiber or tube form is an important parameter in toxic outcome, leading to indirect and direct effects on organisms. Direct mechanical effects were observed in plants, bacteria, and fish, were the CNT pierced and consequently damaged cells. Indirect mechanical effects were observed in algae, crustaceans or insects, where an interaction with the outer body surface occurred, leading to interference with growth and movement. For the assessment of ecotoxicological effects of CNT, the exposure scenario and exposure route has to be derived from the CNT application, use of stabilizers or surface modifications. Here, two scenarios are possible. First, the CNT are kept stable in well-defined test system, where stabilizers may be acceptable. Second, as an environmentally relevant scenario, agglomeration may be accepted. Exposure characterization is an essential part of result reporting, The effect concentrations are above current environmental concentrations and more robust data are needed for future estimates. Future studies with benchmark materials have to clarify uncertainties about exposure/effect relationships. Keywords: environmental organisms, CNT, bioaccumulation Reference: Jackson **P. et al. (2013), Bioaccumulation and ecotoxicity of carbon nanotubes.** *Chemistry Central Journal* 2013, **7**:154

TU176

Carbon nanotubes in aqueous environments – fate and interaction with polycyclic aromatic hydrocarbons

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In this study, the dispersion stability of carbon nanotubes (CNTs) in aqueous media and their interaction with dissolved polycyclic aromatic hydrocarbons (PAHs) was investigated. Five different CNTs, including one single walled carbon nanotube (SWCNT), two multi walled carbon nanotubes (MWCNT-2 and MWCNT-3) and two functionalised MWCNTs (MWCNT-OH and MWCNT-COOH) were dispersed in two types of media; moderately hard reconstituted water (MHRW) and OECD algae growth media (TG 201). Stock solutions (100 mg/L) were prepared by adding CNTs to appropriate media containing Suwannee River natural organic matter (SR-NOM; 20 mg/L) and sonicated. Aliquots of the stock dispersions were spiked into SR-NOM/media solutions to give a final concentration of 10 mg/L and gently mixed. After 24 h of settling, the supernatant containing the remaining dispersed CNTs was taken out for use in the dispersion stability and PAH adsorption studies. The dispersion stability and settling of the CNTs in the two media was determined both in the absence and presence of SR-NOM (20 mg/L) by measuring the concentration of CNTs in the water phase over a period of 14 days (UV-vis, 800 nm). A positive effect on both the concentration of CNTs dispersed and their stability in the dispersion was observed in the presence of NOM for all CNTs. CNT surface functionalisation, especially with carboxyl functional groups, also increased the dispersion concentration. The results indicate that the fate of the CNTs in the environment might vary substantially both with CNT properties and the concentration of NOM. The adsorption of a PAH mixture (naphthalene, phenanthrene, anthracene, fluoranthene, chrysene and pyrene) to the five types of CNTs was investigated. The CNTs were dispersed in synthetic freshwater (MHRW) containing SR-NOM as described above. The PAH mixture was spiked into the CNT dispersions at different concentrations (1-100 μg/L). The CNTs and PAHs were allowed to equilibrate for 5 days before the samples were centrifuged and the supernatant extracted and analysed using GC-MS. Adsorption models were fitted to the adsorption isotherms and the adsorption of PAHs to the CNTs was related to the CNT physicochemical properties. The adsorption of organic chemicals to CNTs, in addition to knowledge about the fate of CNTs in aqueous environments, provides important information regarding the evaluation of their possible environmental impacts.

TU177

Toxicity of PAHs to freshwater algae and Daphnia magna in the presence of carbon nanotubes and natural organic matter

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In the present study, the toxicity of a dissolved polycyclic aromatic hydrocarbon (PAH) mixture in aqueous dispersions of carbon nanotubes (CNTs) containing dissolved natural organic matter (NOM) was evaluated using the freshwater microalga *Psuedokirchneriella subcapitata* and *Daphnia magna*. The adosrption of PAHs to CNTs can influence their bioavailability by reducing the dissolved concentration or expsoure through a different uptake route when adsorbed to the CNTs. The influence of CNT surface area and surface chemistry on PAH adsorption and subsequent toxicity were investigated. Five different CNTs (one single-walled CNT, SWCNT; two multi-walled CNTs, MWCNT; two functionalised MWCNTs, MWCNT-OH and MWCNT-COOH) and a 6 component PAH mixture (naphthalene, phenanthrene, anthracene, fluoranthene, chrysene and pyrene) were used in the study. Stock solutions (100 mg/L) were prepared by adding CNTs to either moderately hard reconstituted water (MHRW; *D. magna*) or OECD algae growth media (TG 201; *P. subcapitata*) containing Suwannee River natural organic matter (SR-NOM; 20 mg/L) and sonicated. Aliquots of the stock dispersions were spiked into SR-NOM/media solutions to give a final concentration of 10 mg/L and gently mixed to homogenise. After 24 hr of settling, the supernatant containing the CNTs remaining in dispersion was removed and an aliquot used to determine the CNT dispersion concentration by UV-vis absorption spectroscopy (800 nm). The bulk of the supernatant was then used for conducting ecotoxicity tests. Initial screening tests for the 5 different CNT dispersions and the PAH mixture were conducted for both *P. subcapitata* (72 hr algal growth inhibition test) and *D. magna* (48 hr acute immobilisation test) to determine their individual LC/EC_{50} values. Algal growth was measured by extraction of the chlorophyll pigments into ethanol and centrifuging prior to analysis by fluorescence (excitation: 420 nm, emission: 671). Finally, the ecotoxicity of the combined CNT/PAH mixtures in SR-NOM were tested. The PAH mixture was spiked into the different CNT dispersions at concentrations above and below the LC/EC_{50} for the PAH mixture. After adsorption equilibration between the CNTs and PAHs had been reached (5 days), the concentration of free PAHs in the sample solutions was determined by centrifugation and solvent extraction of the supernatant. The concentration of PAHs adsorbed to the CNTs was calculated and used to evaluate any change observed in relative toxicity.

Bioavailablity and effects of metals and metal mixtures (P)

TU178

Derivation of ecologically relevant effects threshold concentrations for Pb in marine waters

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In the REACH framework, Pb specific information on environmental toxicity and on environmental exposure/fate for key environmental compartments (water, sediment, soil) was compiled in order to assess the potential environmental risks related to the production and use of Pb in the European Union. Because only few reliable marine chronic toxicity data could be retrieved from literature a testing program was conducted aiming to generate the data necessary to 1) develop a species sensitivity distribution for Pb and 2) derive a safe threshold HC5-50 for the marine aquatic compartment. Consequently, chronic testing using 14 different marine species have been conducted, representing the most relevant taxonomic groups for the marine environment, i.e., crustaceans, molluscs, worms, echinoderms, unicellular algae, higher plants and fish. Where possible, the chronic toxicity experiments were conducted according to internationally accepted standard testing protocols (e.g., EPA, ASTM, ISO). Dissolved Pb levels were measured and

reported effect levels (NOEC, EC10) were based on these measurements. No-effect levels for dissolved lead varied between 9.2 and 1,234 µg Pb/L, i.e. a difference of a factor of 176 between the most and least sensitive species. The mollusc *Mytilus trossulus* was the most sensitive species of the dataset. The least sensitive species were the unicellular algae *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. The HC5-50 that was associated with the best fitting log-normal Distribution that was plotted through the chronic data was 7.0 µg dissolved Pb/L.

TU179

A BLM-normalization software tool for freshwater risk assessment

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The Pb-BLM-SSD-normalization tool is a free resource for anybody interested in using bioavailability-based approaches for assessing the risk of lead (Pb) in the freshwater aquatic environment. The BLM (biotic ligand model) methodology is now considered as state-of-the-art for predicting metal bioavailability because it integrates existing knowledge about metal speciation in the solution surrounding the organism and the interactions between metal ions and competing ions at binding sites on the organism-water interface (e.g., epithelial cells of gill tissue). The development of the tool therefore considers (i) the speciation of Pb, modeled using Visual Minteq and (ii) the competitive binding of Pb2+ and cations (Ca2+, and H+) at the biological membrane (biotic ligand), described as the stability constants for competitive binding of Pb2+, Ca2+, and H+ to the biotic ligand (BL). BLMs for chronic toxicity of Pb are now available for Daphnia, algae, fish and rotifers. The poster will present the development of BLM-SSD software environment enabling the user to derive bioavailability-normalized chronic PNEC values for Pb, by combining BLM based bioavailability normalization of NOECs/EC10s with Species Sensitivity Distribution (SSD) modeling. The 5th percentile hazard concentration (HC5) and its confidence interval are calculated for the lognormal distribution and the best fitting distribution model. Among several user-friendly functionalities, graphical visualization and goodness-of-fit statistics of the SSD fits helps the user to select the appropriate distribution model.

TU180

A comparison of copper Biotic Ligand Models and different Daphnia magna clones and the first steps towards a generic BioAvailability Model (gBAM) for copper.

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When two Biotic Ligand Models (i.e. UGent and HydroQual BLMs) were used to predict chronic copper toxicity for two *Daphnia magna* clones (UGent and CIMM clone), there seems to be a fundamental difference. The UGent BLM accurately predicted toxicity for the UGent clone while the HydroQual BLM more accurately predicted toxicity for the CIMM clone. It was hypothesized therefore that both clones exhibit a difference in their response of copper bioavailability to pH, with the CIMM clone showing a stronger copper bioavailability dependence on pH and also being more sensitive to copper at lower pH levels. This hypothesis was confirmed experimentally in a pairwise clone comparison of chronic copper toxicity at low and high pH. In an attempt to predict chronic copper toxicity data for both clones accurately, a generic BioAvailability Model (gBAM) was developed. This model is structurally similar to that developed for predicting copper toxicity to algae, i.e. copper bioavailability is determined by pH. However, it also includes sodium, magnesium and calcium competition constants, as univariate experiments on *D. magna* have already demonstrated that these parameters have an influence on the bioavailability of copper. The gBAM was able to predict chronic copper toxicity to *D. magna* more accurately (i.e. lower prediction errors) than the original BLMs. Furthermore, a 'read-across' with chronic copper toxicity data of *Brachionus calyciflorus* and *Ceriodaphnia dubia* demonstrated that the gBAM could also be applied to accurately predict toxicity in other species. However, further research and ecotoxicity tests should be conducted to revise the gBAM parameters and to verify the models accuracy and output.

TU181

The influence of salinity and copper exposure on copper accumulation and physiological impairment in the sea anemone, Aiptasia pallida

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Copper is a common pollutant in many aquatic environments, particularly those surrounding densely populated areas with substantial anthropogenic inputs. These same areas may also be exposed to changes in salinity due to freshwater discharge and tidal influence. Although copper is a noted concern, the effects of copper and salinity on symbiotic cnidarians that inhabit near-shore coastal environments are only scarcely studied. The sea anemone, *Aiptasia pallida,* was used in a series of experiments to investigate copper accumulation and physiological effects (i.e. activities of anti-oxidant enzymes) in *A. pallida* at two different salinities (20 and 25 ppt). *A. pallida* were exposed to a control and 3 three elevated copper concentrations for 21 d. Copper accumulation and the activity of the enzymes, catalase, glutathione reductase, gluatathione peroxidase, and carbonic anhydrase were measured in the sea anemones. Photosynthetic parameters in *A. pallida's* symbiotic dinoflagellate algae were also quantified. Over the course of the exposure, *A. pallida* accumulated copper in a concentration-dependent manner; however, higher tissue copper concentrations were observed in the anemones exposed in the lower salinity water (20 ppt), potentially due to the increased copper bioavailability. Physiological impairment was observed as a consequence of both increased copper exposure and decreased salinity. These results clearly demonstrated the influence of two local stressors on a sensitive cnidarian and highlight the importance of characterizing combined exposure scenarios.

TU182

Application of biotic ligand model theory in predicting Ni uptake and toxicity to Enchytraeus crypticus

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Protons and other major cations may inhibit metal uptake and alleviate metal toxicity in aquatic organisms, but less is known about these interactions in soil organisms. The present study therefore investigated the influence of major cations on the uptake and toxicity of Ni in *Enchytraeus crypticus* after 14 days exposure. Ca^{2+}, Mg^{2+} and Na^{+} were found to exert significant effects on both uptake and toxicity of Ni, while K^{+} and H^{+} had no effects. An extended Langmuir model, which incorporated the competition effect of Ca^{2+}, Mg^{2+} and Na^{+}, well predicted Ni uptake by *E. crypticus*. A Biotic Ligand Model was developed to predict Ni toxicity to *E. crypticus*. The predicted $LC50Ni^{2+}$ matched well with the observed values with differences less than a factor of 2. These results suggest that cation competition needs to be taken into account when modelling uptake and effects of metals. The estimated binding constants of Ni^{2+}, Mg^{2+} and Na^{+} on the uptake (S) and toxic action (BL) sites were similar ($\log K_{NiS} = 4.13$ and $\log K_{NiBL} = 3.97$, $\log K_{MgS} = 3.16$ and $\log K_{MgBL} = 3.09$, $\log K_{NaS} = 1.10$ and $\log K_{NaBL} = 1.97$), but for Ca^{2+}, they differed from each other with $\log K_{CaS} = 1.44$ and $\log K_{CaBL} = 3.15$. This indicates that the effect of Ca^{2+} on Ni^{2+} toxicity cannot simply be explained by the effect of competition for uptake sites, which directly inhibits the entry of metal into the organism. Further research on the internal physiological mechanisms of Ca^{2+} interference with Ni^{2+} toxicity is needed.

TU183

Toxicokinetics and toxicodynamics of cadmium in Folsomia candida exposed in a sand-solution matrix

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For soil organisms, bioavailability of metals is mainly governed by exposure through soil pore water. Metals available in the soil solution may be taken up and it is the internal concentration that is supposed to cause effects on the organisms. The main available fraction is the free metal ion which can bind to the biotic ligand sites on the organism thereby causing the effects. Since bioavailability is a dynamic process, time is affecting metal uptake and effects. In the present study, we performed an uptake-elimination kinetics test to study cadmium bioavailability to the springtail *Folsomia candida*. Soil is a complex system to study, because of the dominating effects of the soil solid phase on solution processes and therefore on metal bioavailability. To overcome this complexity, we used test solutions embedded in an inert quartz sand for our exposures. Animals were exposed for 10 days to six cadmium concentrations (0, 0.2, 0.4, 0.6, 1.3, and 2.6 mM Cd) followed by an 11-day elimination period in clean medium. During the uptake phase, animals were sampled at day 1, 2, 4, 7, and 10 while for the elimination phase sampling was after 1, 4, 7, and 11 days. Using a first-order one-compartment model, internal cadmium concentrations were modelled for each cadmium exposure level. Also, cadmium concentrations at the beginning and the end of uptake and elimination phases in the test solutions were measured after 0.45 μm filtration. The results showed a linear accumulation of cadmium in the animals, with uptake rate constants of 0.08-0.46 L kg_{animal}^{-1} d^{-1}. Elimination rate constants ranged between 0.02 and 0.22 d^{-1}. The slow elimination of cadmium caused internal concentrations not to reach steady state levels. Using a toxicodynamics approach, we further studied the adverse effects of cadmium on the survival of *F. candida* over time. Survival-time and LC_{50}-time relationships as well as the relationship between toxicity and cadmium bioaccumulation were investigated.

TU184

Effects of Nickel on Gammarus-induced avoidance behaviour of Chironomus riparius

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Chemicals excreted by predatory organisms (infochemicals/kairomones) can induce defensive responses, e.g., adaptive life history, behaviour or morphology, in their prey (Barry, 2000). The amphipod *Gammarus pulex* has been reported to prey on the aquatic insect larvae (Kelly et al., 2002). In the present study, the predator-induced response of the midge *Chironomus riparius* to the kairomones of *G. pulex* was measured. We also tested the hypothesis that exposure to Ni can influence the *Gammarus* - induced behaviour of *C. riparius*. Avoidance via alternative microhabitat/waters choice by *C. riparius* (3^{rd}–4^{th} instars) was examined in laboratory *y*-maze experiment. Water containing *Gammarus* kairomone (K+) was prepared by culturing *G. pulex* in 200-L tank. Control water (K-) was carbon filtered tap water without *G. pulex*. The results show that the (K+) water was always avoided by the midges (Tukey test, $p< 0.05$). The control treatment of (K-) water against (K-) water revealed no preferences of the midges. In the second experiment, newly hatched larvae of *C. riparius* were maintained for 14 days in the (K+) and (K-) waters. Survival and growth of the midges in (K+) were not significantly different from those of the midges in (K-) water. Feeding rate of the (K+) midges (0.066 ± 0.025 mg/mg/d; mean ± sd; n=4) was higher than that of the (K-) midges (0.049 ± 0.011 mg/mg/d; mean ± sd; n=4) although not significant. Finally, a crossed-design experiment with 2 infochemical levels (K+ and K-) and 6 concentrations of Ni (0-180-320-560-1000-1800 μg/L), was conducted. Each treatment consisted of 3 replicates and 10 newly hatched larvae. After 14 days, no significant effect of both waters and Ni concentrations on the survival, biomass and feeding rates of *C. riparius* was noted (two-way ANOVA, $p>0.05$). *C. riparius* exposed to 1800 μg Ni/L, however, showed no preference to any of the predator levels while the midges exposed to 0 μg Ni/L exhibited their avoidance behaviour. Our results demonstrate that the defensive response of *C. riparius* to the predators could be influenced by Ni toxicity. *C. riparius* and *G. pulex* live together in a wide range of ecosystem and are commonly used species in ecotoxicological tests. Further investigations taking into account their behavioural interaction would increase our understanding of the complex processes and mechanisms affecting the toxicity of contaminated sediment.

TU185

Acute toxicities of 40 rare metals and others with Daphnia magna.

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The rare-metals are used for the electric devices, a car and catalyst recently and the production/the consumption of the rare-metals tend to increase year by year. For instance, a semiconductor constructed in Copper Indium Gallium Selenium (CIGS) is used for the solar panel. Moreover, Indium is also used for liquid crystal panels. The consumption of Indium increased to 33.5 times in current Japan since 1983. Most rare metals, however, are not recycled to take an expense for recycling. Therefore the rare metals may be released in the environment via an e-waste. Nevertheless, the ecological toxic evaluation of the rare metals is not done enough at present. The purpose of my study is to evaluate the ecological toxicity of 40 rare metals (Li, Be, Sc, Ti, V, Cr, Mn, Co, Ni, Ga, Ge, As, Se, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Bi) by biological test using *Daphnia magna*. The biological test was conducted acute immobilisation test with *Daphnia magna*. About some metals which was not soluble in water the highest concentration of the exposure was examined at an upper limit of the aqueous solubility. The concentrations of each metal were analysed by ICP/MS at start and end of test. In the result, The toxic metals with less than 10ppm of EC50 values were Be, Sc, V, Cr, Co, Ni, As, Se, Y, Rh, Ag, Cd, Sb, Os, Pt, Au, Hg and Tl. The metals with moderate toxicity (EC50 value 10-100ppm) were Li, Mn, Cs and Ir. Ti, Ge, Sr, Zr, Mo, Ba and Bi were no effect at 100ppm.Ga, Pd and Ru were affect the D.magna physically. Now, Nb, In, Sn, Te, Hf, Ta, W and Re are analyzing by ICP/MS. The metal toxicity did not relate to an atomic number, a group and ionic radius. There does not seem to be the relationship of the position of the periodic table and the ecotoxicity.

TU186

Toxicity of thorium to various aquatic and terrestrial organisms

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Thorium (Th) is about three to four times more abundant than uranium in the earth's crust and soils contain about 6 ppm on average. In minerals as thorite, thorianite or monazite thorium (Th) is accumulated. Thorium (Th) compounds are highly insoluble in water at neutral pH, i.e., acidic solutions precipitate if the pH changes to neutral and alkali conditions (Langmuir & Herman, 1980; Östhols, Bruno, & Grenthe, 1994). In soils, thorium (Th) has a distinct affinity to clay and organic matter (Thibault, Sheppard, & Smith, 1990). However, thorium (Th^{4+}) may be dissolved in the soil water at elevated concentrations due to anthropogenic discharge (mining) and the presence of various natural chelating agents, such as low molecular weight organic acids or fulvic acids (Chen & Wang, 2007). Little is known about the toxic effects of thorium (Th^{4+}) on organisms in aquatic and terrestrial ecosystems. In order to test the thorium toxicity the radioactive isotope ^{232}Th was used derived from a thorium nitrate solution. A method was developed to reduce the nitrate content of the Th solutions. The aquatic bacterium *vibrio fischeri*, algae *desmodesmus spec*. and the crustacean *daphnia magna* were exposed with Th^{4+} as well as the terrestrial organism *collembola candida*. None of the tested organisms showed any effect when exposed to Th even at high concentrations up to 40 mg/L, thus, no effect concentrations [ECx] in acute or reproduction tests could be observed. The effect of radioactice radiation of ^{232}Th is not considered in our studies because of it´s extremely long half life (14 billion years). Currently we test the mutagenic potential of ^{232}Th in the organisms; results will be presented. Key words: thorium; ecotoxicology; collembola; algae; daphnia;

TU187

A randomization test for statistical significance of non-additive toxicity in metal mixtures

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The toxicity of metal mixtures can be classified as less-than-additive, additive, or more-than-additive, but no widely-accepted test exists for determining the statistical significance deviations from predictions of additive toxicity. Two basic types of additive toxicity are generally recognized: concentration addition (in which the mixture constituents are assumed to act interchangeably when normalized to their respective toxic potencies) and response addition (in which the mixture constituents are assumed to act independently in causing toxicity). We propose a general approach that uses randomization of single-metal toxicity information and mixture-toxicity results, to determine whether interaction coefficients (defined separately for concentration-additive and response-additive toxicity models) differ significantly from zero. The test can be conducted using any spreadsheet-based computer software that allows Monte Carlo-type simulations (e.g., Crystal Ball™, @RISK™). Input information is: means and standard deviations of the probit or logit slopes and intercepts of concentration-response curves (e.g., for mortality) for each of the mixture constituents determined in single-metal toxicity tests, and the mean and standard deviation of the mixture toxicity (e.g., expressed as mortality). Cross-correlations among input variables can also be incorporated into the analysis, and this approach could also be used for other types of chemical mixtures. Example binary Cd-Zn toxicity results demonstrate that with actual “noisy” data, it can be difficult to infer significantly non-additive toxicity even when the results appear to qualitatively demonstrate a strong protective effect of Zn against Cd toxicity (i.e., less-than-additive toxicity). And even with relatively low-variability toxicity data, observed and predicted mortality in a binary mixture must be relatively large (e.g., 20-40%) in order to infer significance at the 95% confidence level. The proposed statistical test will help replace qualitative judgments about non-additive mixture toxicity with quantitative inferences, thus helping to clarify the current uncertainty in this aspect of toxicology.

TU188

Ecotoxicological assessment with Echytraeus crypticus of metal-mine waste polluted soils from Mediterranean Southeast Spain

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The negative effects of mining activities are currently major concerns worldwide due to the disposal of huge amounts of wastes with high levels of metals. In addition, the consequences of mining also affect areas far away from the mining site, when tailings are eroded and wastes transported and deposited in lowland areas. Traditionally, the evaluation of polluted sites is performed through chemical analysis of soil for total and exchangeable metal fractions. However, the latter is not enough to evaluate the environmental risks of polluted areas and the integration of chemical and biological (toxicological) information is necessary to properly asses the ecological risks. The aim of this study is to evaluate the risk of different types of soils polluted by metal-mine wastes through the evaluation of the survival, reproduction and metal bioaccumulation of *Enchytraeus crypticus* (a soft-bodied Oligochaete living in contact with the soil solution and the contaminants present in soil). For this purpose, 6 different types of soils polluted by metal-mine wastes were collected in the mining district of La Unión-Sierra de Cartagena (SE Spain) and in the surrounding areas: Soil 1 (sandy loam texture, pH_{CaCl2}~7.4 and electrical conductivity (EC)~2.2 mS cm^{-1}) and Soil 2 (silty clay texture, pH_{CaCl2}~8.0 and EC~7.6 mS cm^{-1}) were both from a mine tailing; Soil 3 (loam texture, pH_{CaCl2}~6.0 and EC~2.4 mS cm^{-1}) from a watercourse coming from the mining area; Soil 4 (clay loam texture, pH_{CaCl2}~7.2 and EC~0.4 mS cm^{-1}) from a forest close to the mining area; Soil 5 (clay texture, pH_{CaCl2}~7.3 and EC~3.2 mS cm^{-1}) from an agricultural soil near the mining area; Soil 6 (clay texture, pH_{CaCl2}~7.5 and EC~20 mS cm^{-1}) from a salt marsh near the mining area. All soils showed high total contents of metals and metalloids (in mg kg^{-1} d.w.): As ≥ 300, Cd ≥ 7, Cu ≥ 54, Pb ≥ 2000 and Zn ≥ 3000. Toxicity tests were performed exposing *E. crypticus* to the different metal-polluted soils at 20 ^{0}C, according to OECD guideline 220. Survival, reproduction and total internal metal concentrations (As, Cd, Cu, Pb and Zn) were determined after 21 days of exposure. Lufa 2.2 soil (sandy loam texture, pH_{CaCl2}~5.5 and EC~0.1 mS cm^{-1}) was used as a control for animal performance. In addition, to cope with the variation in pH and salinity of the polluted soils, different Lufa soils amended with $CaCO_3$ (to increase pH) and NaCl (to increase EC) were used also as additional controls.

TU189

Insights into heavy metal speciation and bioavailability in soils with the technique AGNES

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AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) is an emerging electroanalytical technique specifically designed to determine free metal ion concentrations in solutions. It has been applied to measure free Zn in Mediterranean seawater [1], river waters [2] and wine and to follow the dissolution kinetics of dispersions of nanoparticles of ZnO [3]. Cd and Pb complexation with humic acid [4] and Pb complexation with hydrophobic, transphilic and hydrophilic fractions of dissolved organic matter of river Seine [5] have also been successfully tackled. AGNES was crossvalidated with Donnan Membrane Technique (DMT) for the determination of free Zn in $CaCl_2$ extracts of 4 Dutch soils [2]. This communication will focus on the application of AGNES technique to assess for the speciation and availability of Zn in soils. Four calcareous soils cultivated with vines are selected for this evaluation. Due to the management practices in vines, metals like Cu, Zn or Mn can be present, and their available levels should be monitored to avoid toxic effects. Free Zn concentrations are determined with AGNES in 4 standard extractions: water, $CaCl_2$, NH_4OAc and DTPA+TEA+$CaCl_2$ (UNE 77315)[6]. Soil properties that condition the fixation or extractability of the metal are evaluated. The correlations between the results obtained for the different extractants and the total and free Zn determined in plant extracts will be discussed. Taking into account the physicochemical phenomena present in the analyses of the soil extracts, this presentation will shed light on the predictive value of the different analytical approaches for assessing the speciation and bioavailability of Zn in these soils. This methodological contribution is expected to be helpful in studying the dynamics of heavy metals in soils. Funding from the Spanish Ministerio de Ciencia e Innovación (CTM2012-39183), from the Comissionat d'Universitats i Recerca de la Generalitat de Catalunya (2009SGR00465) and from AGROTECNIO is acknowledged. References: [1] J.Galceran, C.Huidobro, E.Companys, et al. Talanta 71 (2007) 1795. [2] D.Chito, L.Weng, J.Galceran, et al. Sci. Total Envir. 421-422 (2012) 238. [3] C.David, J.Galceran, C.Rey-Castro, et al. J. Phys. Chem. C 116 (2012) 11758. [4] E.Companys, J.Puy, J.Galceran, Environ. Chem. 4 (2007) 347. [5] B.Pernet-Coudrier, E.Companys, J.Galceran, et al. Geochim. Cosmochim. Ac. 75 (2011) 4005. [6] M.C.Ramos, J. Environ. Manage. 78 (2006) 209.

TU190

PRELIMINARY INVESTIGATION OF THE STATE OF POLLUTION OF OGUN RIVER AT KARA ABATTOIR, NEAR BERGER, LAGOS

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ABSTRACT Intense pollution pressures are exerted on Ogun River, especially at Kara abattoir, by human activities. This river serves as a source of drinking water for the cattle waiting to be slaughtered. It is equally used for domestic activities by the residents along its bank. On daily basis, huge quantities of meat products are transported into Lagos and surrounding states for consumption from the *Kara* abattoir. The consumption of the meat products from the abattoir could pose a great health risk to all consumers. Uptake and accumulation of toxic heavy metals by aquatic organisms from water and sediment are possible. The physic-chemical parameters as well as the heavy metal contents of water and sediments from Ogun River at *Kara* were assessed. Sample digestion was carried out using concentrated nitric acid and the extracts analysed using Atomic Absorption Spectrophotometer. The physic-chemical parameters revealed a high pollution of the river. Water analysis revealed that Cd (0.009 to 0.016mg/L), Cr (1.286mg/L), Fe (0.428 to 2.486mg/L) and Pb (0.109 to 0.109mg/L) exceeded WHO limits. Sediment analysis revealed that Cu (1.51 to 7.04mg/Kg), Zn (4.51 to 43.44mg/Kg), Fe (65.78mg/Kg) and Pb (15.12mg/Kg) exceeded FME limits. Keywords: Abattoir, Ogun River, Heavy metals, Risk assessment

TU191

Concept for the assessment of inorganic UVCB (Substances of Unknown or Variable composition, Complex reaction products or Biological materials) under REACH and CLP

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The intrinsic variability in known chemical (i.e. elemental) composition and the sometimes unknown speciation of the inorganic UVCBs (iUVCBs) raise challenges during hazard and risk assessment under REACH and CLP, so a strategic approach has been developed to cope with both uncertainty (U) and variability (V) The hazard profile of a specific iUVCB depends on its composition: concentration of the chemical elements and speciation, with due consideration of their respective V and U. Therefore, the strategy for the hazard assessment focuses on 1) capturing variability in composition based on knowledge of processes and input materials, 2) defining the ranges in elemental and mineralogical composition, their chemical speciation, where applicable, 3) compiling the hazard profiles of the constituents and 4) classifying the iUVCB based on the combination of the data. It is standard practice for companies to apply sophisticated analytical approaches to determine the elemental concentrations of the constituents given their high economic value. The high V in elemental composition is thus well documented, resulting in a low corresponding U. Speciation is pre-set by the input material and process (e.g. oxidation of sulfides) but often not routinely quantified (U). Furthermore, the metal species present may differ from species released/emitted into the workplace atmosphere. The V in elemental constituents is addressed by selecting a worst-case concentration while the speciation of the constituent is unknown (U), the speciation with the worst-case classification is assumed for the iUVCB classification calculation with the aid of the MeClas tool. The tool makes use of constituent specific information from existing metal registration files and the CLP/GHS mixture rules to derive the iUVCB classification. The tool applies additivity and summation rules to account for the combined effects in hazard identification but allows modifying those when iUVCB-specific information indicates reduced bioavailability of the constituents. For the purpose of risk assessment, the hazards of each constituent are assessed and the DNEL/PNEC values for all the constituents for which a hazard has been identified are considered. All relevant identified constituents, workplace monitoring & environmental release data and metal-specific models are considered in the exposure assessment ensuring that U and V are addressed in a pre-cautionary way. Potential combined effects of the iUVCB constituents are assessed in a tiered way: the first - very conservative - tier is based on the summation of the associated RCRs and can be refined by considering toxicity and mechanistic data (e.g. adding RCRs by target organ for human health). In an effort to avoid a combination of worst cases, before summing up RCRs can also be refined on the basis of statistical considerations.

Modelling of chemical fate and exposure in a regulatory context (P)

TU192

Refinement options of OPS for the estimation of atmospheric deposition of typical large particle-bound substance in colling towersrEFINEMENT OPTIONS OF ops FOR THE

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Atmospheric pollutants have an important impact on public health and the environment. Deposition fluxes in soils, sediment and water determine environmental exposure and also indirectly affect general population. To increase the understanding of the conservative air dispersion model, Operational Priority Substances model (OPS), the deposition patterns of chemicals present in energy production liquid cooling tower systems was investigated. The OPS model was run for various hypothetical emission scenarios for a large particle-bound substance. By varying the emission source characteristics in the model (emission height, emission heat content, heat content…) their impact on atmospheric deposition rate has been examined. Deposition rate encompassed wet and dry deposition based on the release calculation of a point source and is related to a surrounding area to mimic agricultural areas. The deposition averaged over the whole area is used as input for the predicted environmental concentration in soil. Deposition fluxes in the soil compartment have been calculated on the basis of these refined model parameters and further discussed. **References** *De Bruin et al. 2010. Risk Assessment Using EUSES; Refinement Options to Estimate Atmospheric Transportation by its Operational Priority Substances Model (OPS); Hum. Ecol. Risk Assess. Vol 16, No. 5, 945-961. Anonymous – ECHA: Guidance on information requirements and chemical safety assessment Chapter R.16: Environmental Exposure Estimation*

TU193

Evaluating the spatial variability of PAH atmospheric concentrations through precipitation sampling

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Atmospheric transport plays a key role in determining the fate of chemicals, especially when they are emitted directly into atmosphere. Precipitations have been widely used to investigate the atmospheric concentration levels of semi-volatile organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), in different environmental contexts (e.g., urban and rural areas). PAHs mainly originate from the incomplete combustion of fossile fuels, wood and other materials and are thus often found in significant concentrations in the atmosphere of urban or industrial areas. There is a growing interest in assessing the spatial and temporal variability of contaminant concentrations, even in mobile phases such as air and water. Such knowledge would be of particular relevance to assess the effective exposure of ecosystems. Additionally, it would be invaluable in estimating source strengths of emissions, especially when models should be run to evaluate the fate in the other compartments. Due to sampling costs limitations, the investigation of concentrations is usually limited to very few points in time and space, which are then assumed as "typical" concentrations. Therefore, it would be desirable to obtain an array of concentration values on a spatial and temporal scheme in order to evaluate the potential spatial and temporal gradient. In order to do so, a number of sampling campaigns was organized to collect simultaneous samples of precipitations in different points of a grid usualling specially devised samplers. Samples were taken along an urban and rural gradient, extracted using solid-phase micro-extraction (SPME) and analyzed by GC-MS for a series of PAHs. The most commonly found PAHs were phenantrene, anthracene, fluoranthene and pyrene. Samples concentrations were processed using geostatistical techniques and gave a picture of the concentration gradients on a spatial scale. The chemical concentrations measured in precipitations were used as input for a multi-scale fugacity model, composed of dynamic double-layered atmospheric compartment and layered litter/soil, in order to estimate air concentrations and source strength and to investigate pollutant loadings to the soil compartment.

TU194

Long-range transport of e-waste: Part 1. An inventory of the global generation and trans-boundary exports towards non-OECD countries.

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Industrialized countries in the northern hemisphere have generally been considered the major source regions of various industrial organic contaminants (IOCs), such as the polychlorinated biphenyls and selected halogenated flame retardants, because that is where they had been manufactured and more extensively used. Bans on the production and use of some IOCs led to significant reductions in environmental and human contaminant burdens in those countries. Elevated burdens of IOCs are sometimes reported far from these global source regions. While the long-range transport (LRT) of IOCs by air and water to remote areas, such as the Arctic, is well characterized, far less is known about the LRT of IOCs through the trans-boundary exports of e-waste to sub-tropical and tropical regions. Contemporary burdens of IOCs that remain surprisingly high in some regions of Africa and Asia implicate these areas as recipients of obsolete products and wastes containing IOCs from developed countries. A more accurate and complete knowledge about the sources of IOCs which considers LRT of relevant wastes is essential to (i) develop rational control strategies on a global scale, and (ii) better understand and predict the global source-receptor relationships of these contaminants. The main objective of this study is to compile and evaluate existing data on the generation and trans-boundary movement of e-waste in order to develop and present (i) a first quantitative global budget of its contemporary generation, and (ii) to develop scenarios for the global movement on a global scale with emphasis on exports to non-OECD countries within sub-tropical and tropical regions. Patterns, limitations and uncertainties in the resulting budget and scenarios will be highlighted and discussed. This study represents the first step in an overall attempt to evaluate the potential implications of trans-boundary exports of e-waste for global emissions, distribution and fate of IOCs (see Armitage et al. SETAC 2014).

TU195

Long-range transport of e-waste: Part 2. Exploring the potential implications of trans-boundary e-waste exports on chemical emissions, fate and exposures

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Trans-boundary movement of e-waste and the associated contaminants (e.g., polychlorinated biphenyls, halogenated flame retardants, metals) has emerged as an important research topic in the last decade. Several monitoring studies published in the peer-reviewed literature have documented elevated levels of various industrial-use organic contaminants (IUOCs) in the atmosphere near known or suspected e-waste receiving and processing sites in Asia. Surprisingly high concentrations of polychlorinated biphenyls in the atmosphere were also reported offshore of West Africa. Emissions of IUOCs linked to trans-boundary movement of e-waste have implications for chemical fate and exposure at local, regional and global scale. For example, the transfer of e-waste from temperate climates to tropical regions could lead to enhanced emissions simply through temperature-related increases in passive volatilization from open landfill sites. The main objective of this study is to develop emission scenarios for selected IUOCs considering the generation and transport of e-waste and simulate and compare chemical fate and transport using an evaluative modeling approach. All simulations were conducted using BETR-Global 2.0 (https://sites.google.com/site/betrglobal/), a chemical fate model which divides the globe into 288 zones (15° x 15°). This spatial resolution is deemed suitable for assessments at the regional as well as global scale. Breivik et al. (SETAC 2014) present an inventory of the global generation and trans-boundary exports of e-waste towards non-OECD countries, with an emphasis on locations in sub-tropical and tropical regions. This inventory along with the physical-chemical property data of selected IUOCs (e.g., partition coefficients, degradation rate constants) are the key inputs to the model simulations. Model output under various emission scenarios are compared in terms of overall persistence (P_{OV}) at the global scale as well as in terms of long-range transport potential (LTRP) at regional and global scale (e.g., atmospheric deposition of IUOCs in remote regions). The model outputs are also used to assess the potential implications for chemical exposure at regional and global scale under the various scenarios.

TU196

Modelling the climatic evolution of benzo-*α*-pyrene levels over Europe

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The impact of climate change on air pollution is a critical issue. Changes in climate for the past decades can affect the patterns of polycyclic aromatic hydrocarbons (PAHs) by changing the dispersion (wind speed, mixing layer height, convective fronts), deposition by precipitation, dry deposition, photochemistry, natural emissions and concentrations background. Moreover, the availability of BaP observations over Europe is scarce, and only few measurements are available as weekly or monthly averages over this target region. Hence, the use of climate-chemistry transport models to represent the evolution and fate of these pollutants through a multi-scale system can be a very valuable tool to complement and benefit from observations. Therefore, this work relies mainly on the combination of WRF/CHIMERE models, with the addition of the modified EMEP (European Monitoring and Evaluation Programme) emissions. Experiments span the period 1989-2010 and cover the European continent, particularly the areas under EMEP assessment. The atmospheric simulations have a horizontal resolution of 25 km and 23 vertical layers up to 100 hPa, and were driven by the ERA-Interim reanalysis. In order to understand the spatial patterns of PAHs, modelled concentrations of benzo-α-pyrene (BaP) will be evaluated against observations at the monitoring stations belonging to the EMEP network. BaP is arguably the most studied PAH, and the reference for PAH air quality standards defined by the European Commission, setting a limit of 1 ng m^{-3} over a 1-year averaging period is A number of metrics will be used to examine the model performance. For example, the mean bias (B) is a common metric used to quantify the departure between modelled and observed quantities, while the mean normalized bias (MNB) represents a useful measure of the overall over- or under-estimation by the model of the monitored concentrations. These parameters will provide information on the skill of the model to reproduce accurately the dynamics of PAHs over the target area. Moreover, the trends and processes affecting the levels of BaP over the Mediterranean basin will be characterised and defined.

TU197

The climate change induced difference in the multimedia fate of VOCs and PCDDs/DFs as assessed on a monthly basis

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Influence of climatic change (CC) on the environmental fate of 4 VOCs (BTEX) and 7 PCDDs/DFs was assessed using a region-specific multimedia model (KPOP-CC). Two climate conditions were compared, one with meteorological data obtained from climatic modeling for the period of 2000 through 2050 driven by SRES A1B scenario, and the other with the current climatic conditions. To assess the CC influence, monthly average concentrations in air, soil, water, and sediment and inter-media flux were compared for the period of 2041 to 2050. The averages over the 10 year period of monthly average concentrations of VOCs and PCDDs/DFs in air reduce by up to 6% due to CC. The CC reduces the average concentrations of VOCs in soil and water by up to 12%. The average concentrations of PCDDs/DFs in soil, water, and sediment increase by up to 5%, 5%, and 19%, respectively. All the climate change induced (CCI) differences in the average concentrations are statistically significant except those of PCDDs/DFs in water. The maximum monthly average concentrations in air, soil, water, and sediment are

increased by CC up to 43%, 163%, 491%, and 169%, respectively. For both chemical groups, wind is the prevailing factor for the CCI difference in the atmospheric concentration while wet deposition of particle-bound PCDDs/DFs surpasses the influence of wind during wet periods. Increase in rain frequency due to CC contributes more to the CCI increase in the wet deposition than that in the rainfall quantity. In soil, CCI differences in monthly average concentrations of VOCs correspond with those in air because the atmospheric dry deposition of gaseous VOCs is the major input process to soil where rapid degradation does not allow accumulation. PCDDs/DFs are input to soil mainly by wet deposition and accumulate in soil due to their persistence. The concentration of VOCs in water primarily depends on that in air through the dry deposition of gaseous VOCs from air. During wet period, input by surface run-off from soil dominates, hence CCI decrease in the rainfall in autumn leads to the decrease in the VOCs concentration in water. As PCDDs/DFs in the solid surface runoff crucially contributes to their water concentrations, increased rainfall by CC causes the CCI increase of their water concentrations, which leads to the increase of those in sediment. These results suggest that CCI increase in the frequency and quantity of rainfall can significantly raise the risk of aquatic ecosystem.

TU198

Sensitivity analysis of key environmental fate parameters used in PETRORISK version 6.02 for the environmental risk assessment of petroleum substances

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The standard environmental risk assessment framework, under REACH, was developed to consider single compound organic 'substances'. Approaches to assess the environmental risk of substances outside this remit, substances of unknown or variable composition, complex reaction products or biological materials (UVCB's), is less well developed. One group of UVCB's, petroleum substances, can have a large number of constituents, of varying compositions, that make the environmental risk assessment challenging. Several models have been developed to quantify the physical-chemical, environmental fate and ecotoxicological properties of these substances. In particular, PetroTox and the Hydrocarbon Block Method (HBM) tools have been developed, by CONCAWAE, to predict the environmental fate and ecotoxicity properties of petroleum substances. This data has been used to develop PETRORISK version 6.02, which is a standard industry tool used for the environmental risk assessment of petroleum substances, under REACH. In October 2013, ECHA published a review of the environmental and physicochemical methodologies used in the PetroTox and HBM tools. A number of uncertainties about the physical-chemical, environmental fate and ecotoxicological parameters were identified. Inconsistencies in the use of parameters generated from PetroTox and HBM and, their subsequent use in PETRORISK version 6.02, were also identified. This study investigates the potential effect of uncertainty in different environmental fate parameters identified in the review published by ECHA, on environmental exposure. A sensitivity analysis has been conducted using a range of petroleum substances, investigating the effect of variation in the octanol-water partition coefficient (Kow), Henry's Law Constant and degradation rates in sediment, soil and sewage treatment plants (STP). The effect of uncertainty in the bioconcentration factor (BCF) on secondary poisoning and humans *via* the environment has also been investigated.

TU199

Development of environmental multi-media fate model to predict pollutants behavior in coastal area including intertidal wetland

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The coastal wetland serves as a habitat and spawning area for migratory birds and estuary-based marine organisms, and a provider of shellfishes/fishes to people. This area, being ecologically and geographically diverse, is also regarded as one of the most sensitive regions to the global climate-change. The Korean coastal wetland occupies about 2,550 km^2, accounting for 2.5% of inland area, and 83% of which is developed along the west coast where high-tidal range and very shallow slope are established. This costal region is influenced by a number of pollution sources including the freshwater inflow, urbanized/industrialized cities in the region, marine accidents such as oil spills, and atmospheric/oceanic transport.
Nevertheless, the study on the effect of tide on the distribution and fate of pollutants in the multiple media of coastal region has been to date and is scarce in present. The objective of this study is 1) to develop the environmental multimedia fate model (MMFM) to assess the effect of diverse environments to be developed by a large tidal range on behavior and distribution of pollutants and 2) to predict the effect of climate-change on pollutant fate in the coastal wetlands. We verify and calibrate a proto-type MMFM developed, and will present the analysis results (sensitivity and uncertainty analysis) in the meeting.

TU200

Physical-chemical properties and evaluative fate modeling of 'emerging' and 'novel' brominated, and non-halogenated flame retardants

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Following the phase-out, restrictions and tight regulations on the production and use of some brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and more recently hexabromocyclododecane (HBCD), there has been a growing interest in the use of alternative flame retardants (FRs) in materials. Among the chemical substances introduced or considered as suitable replacements are the 'novel' and 'emerging' BFRs as well as a wide group of halogen-free flame retardants (HFFRs), including organophosphorus (OPFRs) and inorganic FRs. Although some of these chemicals have already been used for years, little is known about how they behave and distribute in the environment. Using available physical-chemical property data and state-of-the-art *in silico* tools, namely COSMOtherm, SPARC, EPISuite and multimedia fate models, we perform an evaluative modelling assessment of the likely indoor and outdoor fate of a large number of alternative FRs to give a preliminary indication of their behavior and benchmark it against PBDEs. Special focus is given to modelling the indoor environment since house dust ingestion is regularly viewed as one of the main human exposure pathways for many of these chemicals. Model predictions of the environmental fate of the FRs studied are illustrated with the aid of chemical space plots. An effort to compile a comprehensive dataset of the physical-chemical properties required by the chemical fate models (i.e. the octanol-water and air-water partition coefficients for non-ionic, hydrophobic organic compounds) revealed a serious paucity of experimental data for many of the alternative FRs. Model estimated environmental fate of many of the alternative BFRs is similar to that of the PBDEs due to the similar physical chemical profiles of the alternatives (moderate to high hydrophobicity, low volatility). In contrast, OPFRs are much more hydrophilic than BFRs and this trend is reflected in model estimations of environmental fate. Inorganic FRs have different environmental speciation and partitioning behaviour (i.e. several are ionisable) from the organic FRs. However, it is still possible to provide reasonable estimates of the likely distribution of ionisable FRS in the environment.

TU201

Modelling organic carbon flows and contaminant transport in the Baltic Sea watersheds – impact of future climate and land use change

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Particulate organic carbon (POC) is a major transport vector for many organic contaminants, thus the description of POC advective fluxes between various environmental compartments is crucial in multimedia fate and transport models. Organic carbon export from the terrestrial to the aqueous environment is closely related to climatic conditions and land use. However, the impact of climate and land use change related to the changes in carbon flows and catchment hydrology on contaminant levels in the Baltic Sea has thus far not been assessed. In addition, efforts to dynamically model fluxes of organic carbon in the rivers discharging into the Baltic Sea has so far focused mainly on inorganic and dissolved organic carbon, hence neglecting POC. The total POC fluxes in rivers are dependent on drainage intensity, rainfall intensity and basin slope (i.e. the allochthonous contribution) as well as on the temperature, water flows, watershed geomorphology, and nutrient loading and recycling (i.e. the autochthonous contribution). In this study, we aim to dynamically model the POC fluxes in all major rivers discharging into the Baltic Sea. This is done by implementing the Modified Universal Soil Loss Equation (MUSLE) and an estimation of the litter fall and in-stream production based on land class into the hydrological/nutrient catchment model CSIM. CSIM is used in the Baltic Nest system and calculates the river discharges in all watersheds based on precipitation and temperature. This extended model will be used to assess the changes in POC loads to the Baltic Sea from the various watersheds as a function of future climate and land use change. In addition, the potential consequences of changing POC fluxes for organic contaminant transport to the Baltic Sea via rivers will be assessed.

TU202

PAH and OPAH air-water flux and toxicity before, during and after shoreline oiling from the DWH/BP oil spill

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Biological Response Indicator Devices Gauging Environmental Stressors

(BRIDGES) is a bio-analytical tool that combines passive sampling with toxicity bioassays to provide quantitative measures of the toxicity of bioavailable complex mixtures. Passive sampling devices were used to measure the air gas phase and water dissolved phase at four Gulf of Mexico coastal sites prior, during and after shoreline oiling from the Deepwater Horizon (BP) oil spill in the Gulf of Mexico. Thirty-three polycyclic aromatic hydrocarbons (PAHs), and 22 oxygenated PAHs were quantified, and the presence/absence of 1,182 chemicals were screened for a year May 2010 to 2011. PAH net flux and OPAH flux direction were determined. Prior to shoreline oil PAHs were depositing from air to water. After the oil spill flux changed, and PAHs were volatilized from water to air. The largest sums of PAH volatilized, 5,000 - 10,000 ng/m^2/day ccurred in summer of 2010, at all four Gulf of Mexico sites in 2010. The largest individual PAH volatilization to air was phenanthrene at Grand Isle, Lousiana and acenapthene at a rate of 6,800 ng/m^2/day in Gulf Shores, Alabama in summer of 2010. Toxicity/bioactivity was assessed in the *in vitro* Salmonella mutagenicity assay and *in vivo* embryonic zebrafish developmental model. Spatial and temporal differences were observed in both bioassays and results were site/time dependent. For example, Grand Isle, LA and Gulfport, MS sites showed an increase in mortality observed at 24 hours post fertilization exposure. Overall, Salmonella showed little to no increase in mutagenicity when exposed to Gulf of Mexico air samples. When positive results were observerd there were significant differences between spatial and temporal Gulf of Mexico air samples.

TU203

Degradation in soil of a short hydrocarbon cut: comparison with biodegradation data under standardized conditions

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In order to conduct risk assessment for biocides, agrochemicals and chemicals in different environmental compartments the degradation due to biotic or abiotic processes should be made available. When data originating from studies are not available, alternatives are possible like QSAR, read across or any other extrapolations. In addition when this is not possible, general assumptions are made and are based on worst case situations. The objective of this paper is to demonstrate that in certain circumstances, these general assumptions are not realistic and may lead to misleading conclusions regarding risk assessment purposes. A typical example is given here based on experimental data related to a hydrocarbon solvent. According to REACH guidance (R16)[1] when no data from tests simulating the conditions in soil or sediment are available, the use of screening test data may be considered. The guidance for use of such data is based on the general recognition that for substances with high Kp-values it is assumed a dependence of Kp with of the soil biodegradation half-life. As an example, high Kp > 9000 l.kg-1 leads to conservative degradation half-life for (bulk) soil (DT 50 of 3000 days) for a substances readily biodegradable failing 10-d window. Another approach from US-EPA[2] to getting the required soil and sediment half-lives is to use the recommended values for water and extrapolate. Indeed for screening purposes, it is valid to assume that biodegradation in aerobic surface waters is about as fast as degradation in aerobic surface soil, see Boethling et al. (1995)[3]. Thus DT 50 in soil would be estimated at 5 days for a substance readily biodegradable failing 10-d window. A test for aerobic and anaerobic transformation in soil is being conducted following OECD 307 guidelines. The experimental results will allow discussing the two previous approaches to estimate soil half-life for the substance of interest.

References *[1] Guidance on information requirements and chemical safety assessment Chapter R.16: Environmental Exposure Estimation [2] Interim Guidance for Using Ready and Inherent Biodegradability Tests to Derive Input Data for Multimedia Models and Wastewater Treatment Plants (WWT) Models (9/1/2000) [3] Boethling RS, pH Howard, JA Beauman and ME Larosche. 1995. Factors for intermedia* extrapolation in biodegradability assessment. Chemosphere 30: 741-752

TU204

Antifouling biocides in German inshore & inland waters - How reliable are exposure prognoses of EU scenario models for marinas?

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Numerous laboratory, mesocosm, and field studies have demonstrated adverse effects of selected antifouling biocides in marine and freshwater. A prerequisite for robust modelling of environmental antifouling concentrations released from leisure boats is the reliable inventory of boats and the regional distribution of marinas and other mooring sites. For Germany, such area wide data were lacking so far. Against this background, a comprehensive survey was initiated and funded by the Federal Environment Agency (UFOPLAN 2011, FKZ 3711 67 432) in order to quantify the amount of leisure boats in marinas and other locations in both inland and inshore waters. The census of the number of leisure boats at their berths in German waters revealed a total of c. 206.279, of which 146.425 (71%) boats were located in freshwater, 54.079 (26.2%) boats in brackish waters (S< 18‰), and 5.775 (2,8%) boats in marine waters (S>18‰). The structure and characteristics of freshwater harbours were quite heterogenous and did not match the rigid classification schemes of closed and open harbours. The number of boats at their berth showed a strong season-dependent variation depending on location and sailing season. Areas of high density of leisure boats were identified at the western Baltic coast, the Lower Elbe around Hamburg, the Mecklenburg Lake District, Berlin with its surrounding waters, and Lake Constance. In the second work package, water concentrations of currently used antifouling biocides and some of their specific breakdown products were screened in 50 selected marinas in order to demonstrate the variety of antifoulings occurring in German inshore and inland leisure boat harbours. Further water chemical parameters were analyzed which are relevant for fate modelling. Finally, in a third work package, these measured antifouling concentrations in selected marinas were compared with those calculated by use of the model MAMPEC. With emphasis on freshwater sites the relevance of recent available EU emission scenarios is discussed in view of the actual antifouling exposure in German leisure boat harbours.

TU205

Assessment of river water quality in catchments: Impact of urbanization on particle bound pollutant fluxes

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Transport of many urban pollutants in rivers is coupled to transport of suspended particles, potentially dominated by storm water overflows and mobilization of legacy contamination of sediments. Concentration of these pollutants depends on the mixture of “polluted” urban and “clean” background particles. In the current study, the total concentration of polycyclic aromatic hydrocarbons (PAHs), the amount of total suspended solids (TSS) and turbidity were measured on a monthly basis in water samples from 5 catchments with contrast-ing land use in Southwest Germany and 3 catchments in the Bode Basin in Eastern Germany over up to 1.5 years. In addition, single flood events with large changes in turbidity were sampled at high temporal resolution. Linear correlations of turbidity and TSS where obtained over all catchments investigated and over an extended turbidity range (up to 2000 NTU for the flood samples). Linear correlations were also obtained for the total amount of PAH and suspended sediment concentrations even for very high turbidity or TSS values (> 2000 NTU or mg l-1, respectively). From the linear regressions concentrations of PAHs on suspended particles were obtained - and which varied by catchment. The values comprise a robust measure of the average sediment quality in a river network and may be correlated to the degree of urbanization represented by the number of inhabitants per total flux of suspended particles. The findings are promising for other particle-bound contaminant fluxes (PCBs, phosphorus, and several heavy metals, etc.). Using on-line monitoring of turbidity (e.g. by optical backscattering sensors) mass flow rates of PAH over time could be calculated. Results showed that by far the largest amount of annual PAH loads occur at relative high turbidities > 100 NTU.

TU206

Mixture-toxicity exposure assessment in FOCUS surface water scenarios – Development of a software tool and implementation of a promising risk assessment approach into a user-friendly interface

D. Weber, Eurofins Regulatory AG / Bio V

The registration of plant protection products in the EU requires a standard risk assessment for aquatic organisms, where toxicity endpoints are compared to PEC_{SW} (Predicted Environmental Concentrations in surface waters). The FOCUS model calculations use realistic worst-case scenarios, where pesticides can enter surface waters via spray drift, runoff or drainflow. Simulations result in complex exposure patterns with multiple peaks over long-term periods. An aquatic risk assessment for mixture toxicity is required for formulations that contain two or more active substances. A common known approach is to evaluate combined effects based on TER (Toxicity/Exposure Ratios) for all actives. This approach evaluates the cumulative risk and conservatively assumes a simultaneous occurrence of exposure peaks. However, the FOCUS surface water scenarios often show exposure patterns where occurring maximum concentrations differ in time for active substances, when applied with identical crop/scenario/application timings. A detailed analysis of the ‘realistic worst-case’ exposure profiles for each of the actives is a promising refinement option for critical scenarios. A software tool was developed that allows the user to perform a step-wise evaluation of mixture toxicity in FOCUS surface waters scenarios for formulations containing up to three active substances. The scenarios can be evaluated individually or simultaneously in a first step, resulting in detailed results and visualizations. If scenarios fail the first evaluation step, an in-depth scenario analysis can be performed in a second step, resulting in the identification of relevant key characteristics (*e.g.* maximum peak duration) for the scenario-compound combinations. Detailed results and professional visualizations provide a profound base for decision-making in higher-tier risk assessment. An example for a time-dependent additive mixture toxicity assessment is presented introducing the step-wise approach and explaining the results. Details of the risk assessment approach are presented by Eck *et al*. in the poster presentation: “Time-dependent Mixture Toxicity Assessment for Aquatic Organisms”

TU207

Dynamic modelling of persistence in environmental compartments

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Substances that are Persistent, Bioaccumulative and Toxic (PBT) or very Persistent and very Bioaccumulative (vPvB) can be identified as potential Substances of Very High Concern (SVHC) under REACH. An understanding of how the concentrations of such a substance in an environmental compartment could vary over time is important in assessing their potential impact. A simple mathematical framework has been developed which allows the concentration of a substance in a single compartment over time to be modelled. The key inputs to the model are the amounts of the substance entering the compartment over time, and a rate constant for the loss of the substance from the compartment. Losses in this context may be via degradation or other processes such as leaching or volatilisation. The model allows, for example, assessment of the key uncertainties in the inputs, and also comparison of different future scenarios, such as cessation of all uses versus limited continued use. This is useful in order to predict the success of different risk management options to limit long-term exposure. Impacts on local, regional and continental scale may be considered. This poster reviews the theoretical framework of the model and its practical implementation.

TU208

Placing persistence in context - Looking for the right P criteria

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EU regulation 1107/2009 classifies plant protection products as being persistent (P) by comparison of its half-lives in different environmental compartments to specific triggers for those compartments. This calssification may however grab an indicvidal parameter in a single compartment out of context and neglects plenty of information available on the fate of the compunds. Latest publications of DG SANCO as well as others (eg. Salomon et al. 2013) propose the consideration of relevant dissipation pathways and geometric mean data for persistence assessment. These conclusions indicate the need of placing persistence in context and all available data on the partitioning and fate of substances in the environment shall be considered for the persistence assessment. Moreover, it is a common interest of all stakeholders to define persistence in a manner which reflects latest scientific achievements. A proposal is put forward which considers various methods as potential bases for the P criterion in a more holistic weight of evidence approach, including overall persistence, accumulation potential and the degrading compartment approach. Keywords: persistence, plant protection products, overall persistence

TU209

Spatio-temporal assessment of increased copper contents in vineyard soils due to historical application of copper fungicides - model region results

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The repeated long-term use of copper (Cu) fungicides resulted in accumulation of the persistent Cu in German soils, especially in vineyard soils with a long use history. Observed Cu soil concentrations at such sites now often exceed the precautionary threshold defined for this heavy metal by the German soil protection act. The models for assessing pesticide soil concentrations within the risk assessment procedure under the European plant protection regulation 1107/2009/EC do not fit the issue of accumulation of persistent substances in soils and differing substrate-based background concentrations. Hence, for the estimating Predicted Environmental Concentrations (PECsoil) resulting from the use of copper fungicides alternative approaches are required. Main objective of the presented study is the development and first application of a methodology for a spatio-temporal assessment of copper accumulation in vineyard soils in the historical vine growing areas “Mosel” and “Vorderpfalz” in Germany. In close cooperation between the agricultural services in RLP (Dienstleistungszentrum Ländlicher Raum), the RLP AgroScience and the State Authority for Geology and Mining (Landesamt für Geologie und Bergbau) the consortium funded by the German Federal Environmental Agency (Umweltbundesamt) conducted an extensive literature research and derived different realistic historical Cu application scenarios for distinct use periods. Integrating this information of the historical use of copper fungicides as well as site-specific Cu background concentrations and the duration of viticultural land use was done by a GeoInformation System (GIS). Based on digitized historical topographical maps the expected Cu soil concentrations were modelled for the two regions. These model results were then checked against measured soil Cu concentrations available from official soil survey programs. “Risk maps” indicating expected Cu concentrations at a parcel based scale were generated as final outcome. Based on a classification system, these maps display spatially explicit soil Cu concentrations of the investigated model regions. The modelling results systematically confirm, that the duration of the viticultural land use represents the main factor for copper accumulations in soil due to long-term use of copper as fungicide. The potential for expanding the general approach to other vineyard regions in Germany will be discussed.

TU210

Catchment scale modelling to refine FOCUS scenario based risk estimates of selected veterinary medicines to drinking water

T. Pepper, G. Hughes, H. Lyons, Cambridge Environmental Assessments; P. Marsden, Drinking Water Inspectorate

Human exposure to traces of veterinary medicines may occur through the consumption of drinking water abstracted from surface or ground water sources in agricultural catchments. A recent Drinking Water Inspectorate (DWI) project (70/2/235) used FOCUS scenario approches to demonstrate safe levels of intake for most veterinary medicines, but identified ten compounds where intake may be close to or above the acceptable daily intake. This project (DWI 70/2/286) assessed the potential risk posed by these ten compounds by generating more realistic estimates of exposure through: (i) surveying veterinary practices to refine the estimates of usage (ii) taking account of manure storage, handling and spreading practices, (iii) improved active substance property information and (iv) the use of a catchment-based simulation modelling approach to improve on the FOCUS scenario approach to take into account catchment dilution. Four groundwater and four surface water catchments representative of each animal type (16 catchments) were selected ensuring these were both vulnerable to leaching and runoff while having high animal manure and veterinary medicine pressures. Single integrated medicine usage regimes were calculated from the survey returns for each compound, livestock group and mode of administration. Catchment specific monthly loads were calculated through integrating the usage survey results with the modelled manure production, handling and spreading. For both the surface water and ground water catchments the maximum daily/annual predicted environmental concentrations for each of the representative catchments and compounds were modelled using a catchment modelling framework underpinned by regulatory models (MACRO; PRZM) parameterised using regulatory guidance. The maximum daily surface water and annual groundwater predicted environmental concentrations for each of the representative catchments and compounds did not exceed the acceptable daily intake concentrations for the three human age classes (adult, toddler, infant) for either conventional or advanced water treatment options. The modelled concentrations were typically a factor of several hundred to several tens of thousands below these toxicological thresholds. Based on the results of this study it is concluded that the ten compounds investigated are not expected to impact on drinking water quality under realistic worst case conditions in real world catchments.

TU211

INCA-Contaminants: A dynamic integrated model for assessing chemical contaminant fate in catchments

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Chemical fate models are important tools for scientific investigation, implementation of environmental regulation and management of chemical risk. Traditionally, fate models for generic chemical pollutants have been developed adopting a highly simplistic approach in the definition of the environmental scenarios. This strategy may provide satisfactory results when the scope of the model is to assess chemical fate and distribution over regional (or larger) scales and low time resolution. On the other hand this approach cannot cope with detailing chemical fate at high spatial and time resolution, where the parameters defining the environmental scenarios vary interdependently. Under these conditions, in fact, the model has to be able to detail sudden changes in environmental conditions as precisely as it does for medium-long term smooth changes. We developed a completely new model based on the INCA (Integrated Catchment) model family. The new INCA-Contaminants is able to simultaneously solve the mass balance of water, carbon sediments and pollutants in a soil-stream multi-branched integrated catchment. Key features include: i) high level of detail in scenario description (e.g. multilayer soil and sediment and specifically defined model for different stream conditions ranging from waterfalls to large rivers); ii) high spatial and temporal resolution; iii) simultaneous simulation of an arbitrary number of chemical interacting among them (e.g. to simulate fate of chemical mixtures and cascades of degradation products; vi) kinetically limited multimedia partitioning of chemicals in the multiphase soil, sediment and water compartments; v) ability of simulate the effect of both diffuse and local emission sources as a function of land use, land cover and local precipitation; vi) fully dynamic simulation of fate of contaminant bounded to individual sediment size classes; vii) water column-sediment exchange of pollutants dependent on flow regime. INCA-Contaminants will be used to predict the influence of local to regional scale perturbations on exposure and occurrence of chemical pollutant mixtures in soil, sediments and surface water due to anthropogenic and/or natural forcing (including land use change, climate change, environmental management, socioeconomic changes, regulation etc.).

TU212
How to estimate application dates for FOCUS surface water scenarios based on plant growth stages?
C. Gaviria, Environmental Fate Modelling; C. Hoerold, Rifcon GmbH
In order to calculate predicted environmental concentrations (PEC) in surface water with the FOCUS model shell SWASH it is necessary to define dates for the intended application for each scenario and crop. No guidance describing a uniform procedure to find adequate application dates based on BBCH codes provided by the GAP has been implemented until now. In SWASH the crop parameters describing the scenarios only include specific dates for the growth stages 'emergence', 'maturation' (for R-Scenarios) or 'max. leaf area development' (for D-Scenarios) and 'harvest'. Information about intermediate growth stages is not provided (FOCUS 2012). Therefore the choice of adequate dates is subject to the user's best of knowledge and can vary significantly leading to different PEC values calculated by SWASH. The aim of this study was to implement a tool which provides application dates for FOCUS surface water (SW) scenarios and crops based on BBCH codes, minimizing the influence of the different user's choice, reducing a time-consuming procedure and being reproducible. The application dates were estimated on the basis of the data implemented in the software *AppDate2.0a[1],* developed by Klein (2013) for groundwater (GW) (FOCUS 2012). Matching SW and GW scenario calendar dates were chosen accordingly. Following Klein's (2013) procedure, missing dates were calculated by linear interpolation between the dates of the main growth stages 'emergence' (BBCH 09), 'maturation' or 'max. leaf area development' (BBCH 60 for all crops, except for cabbage, carrots, onions and sugar beet, where this date corresponds to a BBCH code of 40) and 'harvest' (BBCH 90 for all crops, except for cabbage, carrots, onions and sugar beet, where this date corresponds to a BBCH code of 50). Finally, a user-friendly tool which calculates the respective dates for each FOCUS scenario and crop by entering the BBCH code and the crop type was developed in EXCEL. Besides the absolute application date, this tool also proposes application windows for SWASH. This tool is adjusted primarily for the estimation of application dates needed for SWASH and therefore it is not applicable to other models. However, this tool amends discrepancies between different user's choices and facilitates the selection procedure of the application dates based on BBCH codes. [1]Klein (2013): AppDate2.0.a-Estimation of application dates based on crop development. Frauenhofer-IME, D57392 Schmallenberg

TU213
New Drift Deposition Inputs for Modelling Applications of Herbicides in Orchards and Vineyards
R. Jackson, Dow Agrosciences / REgulatory Sciences; L. Fogg, ADAS UK Ltd / Cambridge Environmental Assessments
Currently, the FOCUS surface water modelling procedures do not include specific spray drift deposition inputs for applications of herbicides in orchards and vineyards. Instead, the values for field crops, which are derived from tractor mounted boom sprayers, are often used as the default. However, the procedure for applying herbicides in orchards and vineyards is very different from field crops. Herbicides are most often applied as a targeted spray onto the strip of soil beneath the trees or vines using a low boom with one or two nozzles mounted on a small tractor. Often, there is a need to minimise contact between the spray and the tree or vine to avoid phytotoxicity. Two studies have been conducted in order to derive percentage spray drift values for herbicides in orchard and vineyards that can be used in the FOCUS surface water models in order to give realistic drift inputs to adjacent surface water bodies. In the first study, % drift deposition of a herbicide product was measured downwind from a 1 m wide bare ground strip that was treated with a hand held sprayer with two nozzles using travel speed, height and pressure that simulated realistic application conditions. Nine separate spray applications were made. In the second study, applications of a tracer dye were made in one orchard and one vineyard using commercial equipment and methods. A total of 19 applications were made with triplicate measurements for each application. Spray drift curves were plotted and the 90th percentile spray drift deposition values were calculated for distances up to 15 m from the treated area. The results showed that spray drift deposition was more than an order of magnitude lower than the standard FOCUS values for field crops.

TU214
Linking exposure models to regulatory and sustainability driven frameworks
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The assessment of exposure of chemicals to environment and to human via the environment is of major concern for policy and industry and ultimately benefits all citizens. Currently many different types of exposure models are available. However, not all exposure models are designed for the same goal. Exposure assessment of chemicals depends highly on the context. In order to allow a comparison between and selection of exposure models, a transparent and objective framework based on a comprehensive list of scoring factors was developed. A comprehensive list of criteria was set up to structure the characteristics of the exposure tools. These criteria were divided into two categories: general and framework/context specific criteria. The general criteria were transferred into questions and were scored on importance by an expert panel. The framework specific criteria, which are highly context specific, received a score based on their applicability and importance in a certain type of framework. The following regulatory and sustainability driven frameworks were considered: REACH regulation, Plant Protection Products Regulation, Biocidal Product Regulation, environmental/spatial oriented directives (e.g. Water Framework Directive), Food oriented directives (e.g. Food Supplement Directive), product or process certification, site specific impact assessment and sustainability assessment. After scoring the criteria, a list of exposure models (such as EUSES, CalTOX, QUASI, 4FUN, USETOX, GREATER, PEARL, etc.) were evaluated using the Multi-Criteria Decision analysis (MCDA). The MCDA approach is a decision support tool, which allowed us to rank the different exposure models per framework. In conclusion, this methodology is ideally situated to determine the strengths and weaknesses of exposure models and to determine the suitability of a model towards a certain type of framework.

TU215
Modelling concentrations of PPP in ground water after spray application to hop
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Currently the the risk envelope approach in the core assessment to minimise the number of individual uses and maximise the value of the core assessment is discussed by applicants and authorities. With respect to ground water calculations, the possibility for a reasonable grouping of crops is supposed to be limited (SANCO/11244/2011). This could be confirmed by several test calculations (e.g. Gimsing et al. 2013, Nickisch & Seiterle-Winn 2013). According to the results of the test calculations it seems to be essential to calculate the use in each crop separately. However, the number of available crops in the FOCUS ground water models is limited. For example hop is missing though at least nine noteworthy growing areas can be found in the EU. Moreover, hop is a special crop with an extensive leaf development greater than for all other crops implemented in the FOCUS models. Therefore, we tested the influence of the leaf development on ground water concentrations. Calculations were conducted for the FOCUS scenario Kremsmünster which reflects best the soil & climate conditions of the hop growing regions in Europe. Two application dates were tested: emergence and 60 days post-emergence. Emergence and harvest dates for hop were set to the FOCUSsw application dates (15th April, 1st September). Leaf area index values (LAI) were derived from evaluation of field experiments with three hop varietys at three BBCH stages (37, 55 and 75) conducted by the LFL ("*Bayerische Landesanstalt für Landwirtschaft"*). Results showed that PECgw deviated considerably between the hop varietys. This can be traced back to the variation in LAI (3.7 – 9.0) and thus in interception. Comparisons between the PECs for different crops considering a relative application date indicated that hop can't be covered by a surrogate crop. Remarkably, by using the hop emergence and harvest dates for the FOCUS crops, the influence of the crop is mainly driven by interception; other parameters have only a minor influence. Calculations affirmed that a risk envelope for PECs in ground water is not trivial. However, it could be shown that the main drivers next to substance parameters are the application date and the soil load. Thus, a conservative tier 1 risk estimation for hop can be done by neglecting crop interception and selecting a FOCUS crop with constant high LAI values like maize or sugar beet in combination with the hop emergence and harvest dates.

TU216
The plant uptake factor (PUF): An update on ongoing activities at EU level
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The EU registration processes require the assessment of the leaching potential of an active ingredient and its metabolites of plant protection products (PPP) to groundwater (GW) as described in FOCUS (2000, 2009). Certain amounts of active ingredients as well as metabolites can be taken up by plants via the root system and are no longer available for leaching processes. For GW simulations using FOCUS-PELMO or PEARL in the EU registration procedure a PUF can be considered to describe this process. Recent analyses showed that using a PUF has a high impact on reducing the GW entries (Bonath et al., 2013 and EFSA Journal 2013;11(6):3291; p 9-10). Therefore the importance of applying a reliable PUF for the authoristation of a PPP is very high. In its opinion on the FOCUS GW report (FOCUS, 2009) EFSA recommends that a study design for the experimental

determination of the PUF should be developed (EFSA Journal 2013;11(6):3291; p 9-10). Therefore representatives of EU member states from university, regulatory authorities and industry joined a workshop held in York in September 2013. Here the scientific experts discussed about an appropriate handling and experimental determination of the PUF within the zonal registration process. As a result of the workshop it has been agreed that the different parameters influencing the PUF and interlaboratory variability have to be investigated in more detail in future ring tests. An update regarding most recent activities in this context will be given in the poster.

TU217
Lysimeter studies and inverse modelling as higher tier options for groundwater risk assessment?
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In the national authorisation of plant protection products in Germany the groundwater risk assessment at tier 1 and 2 is based on simulated leaching concentrations using the FOCUS Hamburg scenario of FOCUS PELMO 5.5.3. Thereby, parameterisation of the degradation and sorption behaviour of active substances and their metabolites is performed according to the recommendations of Holdt et al. (2011). At tier 3, measured leachate concentrations from outdoor lysimeter studies are generally accepted as higher tier endpoints. This is mainly justified by comparable soil and climate conditions in the FOCUS Hamburg scenario and during lysimeter experiments. Further investigations using the methodology of inverse modelling are ongoing to clarify the main critical point, how the short duration of lysimeter studies in combination with a single application affects the prediction of the leaching behaviour. The outcome of the analysis shall indicate under which conditions outdoor lysimeter studies can be evaluated as endpoint or as process studies for active substances and metabolites. First results show limitations of the methodology of inverse modelling of lysimeter studies. Based on these results, the technical requirements on outdoor lysimeter studies in order to perform inverse modelling with FOCUS PELMO 5.5.3 as well as the opportunities and limits of transferability of the outdoor lysimeter conditions to other conditions are discussed. Furthermore, a proposal, how inverse modelling of lysimeter study results could be adequately considered for future regulatory decision making in the context of groundwater risk assessment is presented.

TU218
Wash-off implementation in FOCUSgw models
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Foliar wash-off is the process by which pesticides applied to leaf surfaces may be removed by rainfall after application and enter the soil. Dependent upon compound properties and application timing the inclusion of wash-off can influence the outcome of PEC groundwater (PECgw) calculations. Recent regulatory interest in the wash-off process has suggested that wash-off from plant surfaces be included into the leaching evaluation of foliar applied pesticides (EFSA, 2012 & 2013). We investigate the feasibility of including foliar wash-off into the groundwater assessment with the current parameterization of the FOCUS models. We present a detailed technical analysis of the wash-off parameterization in the four FOCUSgw models PEARL, PELMO, PRZM and MACRO. The analysis includes the FOCUSgw reports, official model documentation, model source code (where available) and extensive model simulations under controlled conditions. Model developers were also contacted directly to answer particular questions. The results demonstrate that the existing FOCUS models use different approaches to simulate the wash-off process and need different parameterization from experimental data. Without care severe deviations in the model calculation of wash-off can result between models. Examples are presented and discussed. This work highlights the need for a model harmonization with regard to parameterization and implementation of wash-off if the level of model harmonization achieved by the FOCUS Groundwater group is to be maintained. Some options and potential strategies to overcome the limitations of the existing models are presented and discussed.

TU219
Coupling leaching simulations with groundwater transport models in the evaluation of monitoring data for plant protection products.
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A key aspect of the evaluation of groundwater monitoring data for plant protection products in targeted or prospective monitoring studies is demonstrating that a hydraulic connection exists between fields treated with the target substance and the monitoring well at which samples are taken. Furthermore, it is important to assess to what extent measured concentrations at the well reflect the groundwater concentrations directly beneath treated fields and the effect of factors such as dilution. In such monitoring studies it is usual to determine the groundwater flow velocity and flow direction and identify fields that lie on the flowpath to the well. Once relevant fields have been identified, previous applications of the target substance can be documented, or, in the case of prospective monitoring, applications for the purposes of the monitoring study can be planned. Once it is known where and when the target substance has been applied, leaching concentrations below treated fields can be calculated quite straightforwardly using leaching models such as PEARL with site-specific data. However, to establish the link between these concentrations and those measured at the monitoring well, in addition to the hydrogeological characterisation of the monitoring site a further step may be supportive. It is in this context that groundwater flow and transport models can be used, taking the output of the established leaching model as an input to simulate concentrations at the monitoring well, considering the hydrogeological circumstances and potential dilution or dispersive processes. For groundwater monitoring data from a study targeting the metabolites of a widely used herbicide, groundwater models coupled with the leaching model PEARL are used to demonstrate the link between documented product applications and measured concentrations at monitoring wells in two different hydrogeological settings. For a monitoring site in Germany with a shallow, unconsolidated sedimentary aquifer the groundwater flow and transport model FEFLOW® is used to simulate metabolite transport to the monitoring well along a 2D vertical profile. For a second monitoring site in France, where a captured natural spring in a shallow fractured aquifer is sampled, a pragmatic mass-balance modelling approach is used to calculate metabolite transport to the monitoring point. In both cases the models can effectively demonstrate the link between product applications and measured concentrations.

TU220
COMPARISON OF MEASURED AND MODELLED GROUNDWATER CONCENTRATIONS OF CHLORIDAZON IN SWITZERLAND
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The concentration of pesticides in groundwater bodies in Switzerland is monitored within the framework of the Swiss NAQUA groundwater programme of the FOEN (Federal Office for the Environment). In 2011 the concentrations of pesticide active substances exceeded the protection goal of 0.1 µg/L at 2% of the more than 500 NAQUA monitoring sites (TREND and SPEZ module). The Chloridazon metabolites Desphenyl-Chloridazon (5-amino-4-chloro-3(2H)-pyridazinone) and Methyl-Desphenyl-Chloridazon (5-amino-4-chloro-2-methyl-3(2H)-pyridazinone) were the substances recorded most frequently in concentrations exceeding 0.1 µg/L. For several NAQUA monitoring sites in Switzerland the measured concentrations for Chloridazon and its metabolites in the year 2011 are available. The aim of the present assessment is to compare the monitoring results with the groundwater modelling results for the FOCUS scenarios Hamburg, Kremsmünster, Chateaudun and site-specific modelling locations and additionally, to set them into regulatory context. The PEC groundwater calculations are going to be carried out with the groundwater prediction model FOCUS-PEARL 4.4.4. For every groundwater monitoring location a specific PEARL scenario is created, based on on-site soil (e.g. soil texture, bulk density, oc-content) and climate data (e.g. min/max temperature, precipitation, global radiation) in daily resolution for the time-span of the monitoring campaign. Three different types of soil hydraulic pedotransfer functions are tested. The respective results will be compared to measured soil conditions to determine a simulation method that represents realistic field data the best by using a goodness-of-fit indicator (sum of squared errors between simulated and estimated volumetric soil moisture and measured and simulated soil temperature). The PEARL simulations are conducted for the FOCUS crop "sugar beets", since only products containing the active substance Chloridazon for the use in fodder and sugar beets are registered in Switzerland. A pre-emergence application and the maximum registered application rate in Switzerland of 2.6 kg/ha are also considered for the simulation. Since the measured pesticide concentrations in groundwater are only representing a few time-points in the temporal concentration sequence of Chloridazon and its metabolites, the measured values are set in context with the complete modelled concentration gradient for the substances for a comparable depth during the investigation period.

TU221
Adsorption coefficient of active pharmaceutical ingredients to sewage sludge: progress in measurement and prediction
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Over the past forty years concerns over the presence of active pharmaceutical ingredients (APIs) in the environment have grown considerably. Some APIs can be effectively biodegraded in waste water treatment plants but others can be sorbed onto sludges that are often subsequently used as fertilisers or disposed of to landfill. This work aims to understand how a given API will be distributed between the aqueous and solid phases within a treatment plant which is important to be able to make accurate risk assessments. A sorption test to measure the partitioning (determination of the K_d value) of an API in sewage sludge is available (OPPTS

835.1110), but is long and fastidious. Predictive models for K_d based on log K_{ow} have been developed for soil and extended to sewage sludge. These models are optimised mainly for neutral organic chemicals, and only a few consider ionic substances. This work compares the performance of these soil-based models on a range of APIs, including ionisable compounds, and assesses their application for ionic and non-ionic pharmaceuticals into sewage sludge. It also explores other predictive models based on molecular descriptors obtained from API structure. These models provide improved predictions over previous models based solely on log K_{ow}. In addition, a new solid-phase extraction screening method was developed to rapidly measure K_d and its performance has been evaluated against K_d values obtained with the OPPTS method and values published in the literature. This rapid method allowed the measurement of additional K_d values for APIs that were not available in the literature and these were used to further validate the new molecular descriptor-based models.

Sorption and bioavailability of organic chemicals: mechanisms and applications in innovative remediation (P)

TU222

Sorption and remediation of halogenated organic pollutants from contaminated water sources using polyphenol-functionalized magnetic nanocomposites

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The focus of detailed research in proper nutrition has resulted in discoveries that extend beyond healthy living and diet-related chronic diseases and enter into the realms of risk assessment and remediation. One example of this is in the use of various bio-molecules and antioxidants (e.g. polyphenols, epicatechin in tea extract, etc.), which have been found to both bind persistent environmental pollutants and modulate their toxicity. This binding affinity can be utilized for biomimetic approaches for the capture, detection and remediation of polychlorinated biphenyls (PCBs) using surface-functionalized magnetic nanoparticle-based technologies. Iron oxide magnetic nanoparticles (MNPs) were functionalized with quercetin multiacrylate (QMA) and curcumin diacrylate (CDA) polyphenolic polymer matrices with high affinity for organic pollutants. This platform allows for the specific binding of chlorinated organics from contaminated drinking water sources, the rapid magnetic separation of bound organics, and the thermal destabilization of the polymer matrix for contaminant release and material regeneration. Particles were characterized via TEM, DLS, FTIR, and TGA; chlorinated organic pollutant (i.e., PCBs) binding studies were performed to determine binding affinity and capacity, as well as optimal binding kinetics. LC-MS/MS analysis demonstrated that the functionalized MNPs effectively bound PCBs with the addition of QMA and CDA resulting in greater affinity. An alternating magnetic field was used to heat and destabilize the PCB binding in the polymer matrix leading to pollutant release from the particles, and the percentage of uptake and release was determined. Repeated PCB binding/release was performed to determine MNM stability and reusability. This work provides a rapid, simple, non-toxic platform for pollutant removal from contaminated water sources in highly industrialized nations as well as in the developing world.

TU223

Impact of activated carbon and biochar on biodegradation of crude oil in produced seawater on off-shore oil platform

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In the world, waterflooding of oil reservoir and fracturing of shale gas reservoir generate 38 million m^3/day of produced water in 2012 and by 2020 production is expected to grow by 21%. On a basis of 50 ppm of crude oil in produced water, about 2000 m^3 of crude oil are discharged in the environment every day. A single off-shore oil platform discharges to the sea 500 to 25 000 m^3 of produced water every day. Therefore it is critical to improve waste water management on off-shore oil platform so that efficient and cheap water treatment forms can be developed. As classical wastewater treatment technologies are not suitable for off-shore oil platform, an approach based on biological aerated filter (BAF) made of polyvinyl alcohol and 1% (w/w) of activated carbon (BAF-AC) or biochar (BAF-biochar) was developed in this study. Activated carbon (AC) and biochar are applied to contaminated matrices (i.e. soil, sediment, water) to strongly sorb hydrophobic organic compounds (HOCs), reducing their freely dissolved concentrations and limiting bio-uptake and toxicity. However, a number of studies have demonstrated that biodegradation of HOCs still efficiently occurs even in presence of small amounts of AC or biochar. The aim of this new BAF technology is to quickly ($t < 1$ day) reduce oil concentration in produced water to 20 ppm by both biodegrading and sequestrating crude oil. This study investigated the abiotic desorption of crude oil from the BAF-AC and BAF-biochar, and compared this to the biodegradation of the sorbed crude oil. The total abiotic desorption ($t = 24$ days) of crude oil from the two BAFs in different sterile aqueous solutions (artificial seawater, artificial seawater plus fertilizer, and artificial seawater plus broth) to an infinite silicone sink made of silicone O-ring was measured. The total amount of crude oil desorbed was lower for BAF-AC compare to BAF-biochar. This was compared to the biodegradation ($t = 3$days) of crude oil sorbed to the soil amendments in artificial seawater plus fertilizer and artificial seawater plus broth with *Pseudomonas putidas* K12. Crude oil was almost completely degraded in all treatments. Therefore, a low abiotic desorption of crude oil does not lead to a low biodegradation. Our results suggest that adding AC and biochar to BAF can increase crude oil sequestration without affecting the biodegradation of contaminants in seawater.

TU224

Dehalogenation of hexachlorobenzene at environmentally relevant concentrations by novel laser-ablated Pd(0) nanocatalysts

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As one of the persistent organic pollutants (POPs), hexachlorobenzene (HCB) is known to be persistent, bioaccumulative, and toxic. Due to insignificant physicochemical elimination and slow microbial degradation, its half-life in environmental media exceeds decades. However, under reductive conditions, a complete dehalogenation of HCB to benzene could be investigated in the presence of metal (nano)catalysts including elemental palladium and iron. HCB concentrations which have been used in most previous studies were in the mg L^{-1} range although its water solubility is about 6 μg L^{-1}. Furthermore, deployed Pd(0) concentrations often reached the g L^{-1} range, which is not appropriate in terms of intended resource efficiency. Within this study, the dehalogenation of 3 μg L^{-1} HCB was studied in aqueous nanoparticle suspensions in the presence of very low concentrations (μg L^{-1} range) of novel laser-ablated Pd(0) nanocatalysts. All experiments were carried out in a micro reaction system under anoxic conditions containing hydrogen as electron donor. HCB and its transformation products were detected using GC/MS. The size of Pd(0) particles was monitored by nanoparticle tracking analysis (NTA). More than 95 % of HCB were completely dehalogenated within minutes, as confirmed by the detection of non-chlorinated benzene. Due to the fast reaction kinetics, no intermediate products could be detected. However, the process could be slowed down by using inhibiting reaction conditions. This enabled the detection of intermediate products and thus elucidated the dehalogenation pathways. With this approach, the dehalogenation potential of different nanocatalysts can be easily estimated, providing an additional parameter for the characterization of nanoparticles. The fast dehalogenation of POPs by nanocatalysts could be a prospective application within remediation/water treatment. The identification of the transformation pathways provides insights in understanding the basic processes of nanoparticle catalyzed dehalogenation reactions.

TU225

Assessing contaminant bioavailability in sediment using direct and passive sampling methodologies

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To evaluate the impact of lipophilic and/or bioaccumulative compounds in sediment, one of the focal point is the study of contaminant bioavailability. Microbial fauna and benthic macroinvertebrates are the first link of the demersal/benthonic food chain potentially affected by pollutants; therefore, it is important to rely on robust and sensitive measurement tools to quantify bioaccumulation and biomagnification processes. The poster will present advantages/disadvantage of different methodologies adopted in several ENVIRON projects to assess contaminant bioavailability in freshwater and marine contaminated sediment sites. In details, methods and results of three different approaches will be presented: 1. in situ sampling of macro-invertebrate fauna, to understand the relationship between benthic organisms and sediments; 2. bioaccumulation tests performed in laboratory on field collected sediments, in order to determine Biota-Sediment Accumulation Factor (BSAF) under controlled conditions; 3.surveys through the use of passive samplers at various depths along water column and in sediments, in order to get an accurate understanding of the exposure dynamics. We will present a brief discussion about the usefulness/advantages of these techniques relating to aim and scope of the different phases of contaminated sediment site management (characterization, Ecological Risk Assessment and monitoring phases before/post remediation).

TU226

Improving bacterial transport and bioavailability by the combined effects of dissolved organic matter (DOM) and bacterial tactic responses

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There is an increasing interest of improving transport of microorganisms in soils and subsurface environments because the attachment of cells to the solid matrix is a very common restriction in bioremediation scenarios. In this study, the mechanisms involved in the improvement of bacterial transport by dissolved organic matter (DOM) with potential use in bioremediation have been investigated. We have assessed the effect of different sources of DOM (sunflower root exudates and humic acids, both at 16 mg L^{-1} and~130 mg L^{-1} TOC) on the transport of the naphthalene-degrading strain *Pseudomonas putida G7* trough sand-filled columns. Root exudates represented a fresh source of DOM, whereas humic acids were representative of processed organic matter. We also studied bacterial deposition on the sand surfaces through batch adhesion experiments. The tactic response towards exudates and humic acids, studied as the possible enhancing mechanism for bacterial transport, was characterized with capillary assays and by computer-assisted motion analysis of motility behavior. We observed that exudates promoted bacterial transport to the same extent and they also triggered a chemotactic reaction, independently of the TOC used. However, humic acids only enhanced the transport at the higher TOC and did not cause chemotaxis in *P. putida G7*. We propose that a modified motile behaviour due to tactic responses can be added to the positive effect of DOM on bacterial transport due to physico-chemical mechanisms. In addition to this, the mobilization of contaminants through interaction with DOM can lead to an increased bioavailability of PAHs in soils, thus causing a concomitant increase in biodegradation rates.

TU227

Promoting microbial life at the aqueous-interface of non aqueous phase liquids (NAPLs): a low-risk strategy to enhance biodegradation of sparingly bioavailable PAHs

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Polycyclic aromatic hydrocarbons (PAHs) associated to hydrophobic NAPLs are known to be poorly available for microbial mineralization. Here, we aim to promote biodegradation of phenanthrene associated to a NAPL (constituted by fuel and heptamethylnonane) by the colonization of the aqueous-NAPL interface. The soil bacterium *Mycobacterium gilvum* VM552 was used as phenanthrene degrader, while different conditions for the microbial mineralization were established. It was found that the interface colonization of *M. gilvum* VM552 was influenced by diverse factors such as the balance of dissolved nutrients and oxygen, the physical influence of aqueous flow dynamics, the attachment and biofilm formation on hydrophobic surfaces, and the rate of chemical partitioning from NAPL. Based on our observations, the balances of dissolved nutrient in either absence or presence of mycelial networks produced by different oomycetes (*Pythium aphanidermatum* and *Pythium oligandrum*) were a key factor for the bacterial colonization. Interestingly, the bacterium was able to colonize better the aqueous-NAPL interface in static condition than in aerated conditions. In addition, the biomass of the oomycetes applied in the aerated system affected the bacterial colonization through a competition for dissolved oxygen demand. We conclude that the colonization of the aqueous-NAPL interface can be a low-risk strategy to increase the bioavailability of PAHs and to promote their on-site biodegradation.

TU228

Simulation and measurement of bacterial growth on low soluble phenanthrene substrate

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The metabolism of low soluble substrate is limited by dissolution and substrate availability and can hardly be determined in a common chemostat. We developed a numerical model that calculates simultaneously dissolution kinetics of such substrate, metabolism (Michaelis-Menten kinetics) and microbial growth (Monod kinetics with decay term) for the dynamic case. Experiments on the degradation of phenanthrene by and the growth of the three degrader strains *Novosphingobium pentaromativorans* US6-1, *Sphingomonas* sp. EPA505 and *Sphingobium yanoikuyae* B1 were used to determine kinetic parameters as input for the model. Phenanthrene (Pht) in acetone was added to 10 mL test vials. The nominal initial concentration of the suspensions was 10, 25, 50, 100, 200 and 400 mg/L. Pht was present as slowly dissolving microcrystals. This provided non-limiting conditions for the growth of the degrader strains over several days. Total Pht concentration and protein were tracked over 6 to 12 days. In all replicates, Pht was completely metabolized, and biomass increased rapidly, more at higher initial concentration, but decayed when Pht was depleted. The model was fitted to the test result in order to determine the rates of dissolution, metabolism and growth. The outcome shows that the three bacterial strains have similar efficiency, with vmax-values of 12 to 18 g bacteria dw / g substrate / d, yields of 0.21 g/g, maximum growth rates umax of 2.5 to 3.8 1/d and decay rates of 0.04 to 0.05 1/d. Simulations with the model show that i) retainment in crystals, NAPL or by sequestration compete with biodegradation, since molecules remaining non-dissolved cannot be degraded; ii) the conditions for bacterial growth (i.e. dissolution flux and resulting chemical activity of substrate) are more relevant for the final state of the system (both concerning number of degraders and time-course of substrate concentrations) than initial biomass; and iii) the desorption flux regulates the turnover when the substrate source is in solid state or present in sequestered (aged) systems. Provided the equations describe correctly the kinetics, the calibrated model can be used to simulate bioavailability, biodegradation, persistence and treatment options in real systems, such as PAH-contaminated soils.

TU229

An energy-efficient and simple bioremediation technique for oil-contaminated soil clean-up by just spraying a versatile foam

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Landfarming is a widely used remediation technology for oil-contaminated soils. Excavated contaminated soils are spread on the ground surface and tilled periodically for aerobic biodegradation. However, landfarming of fuel-contaminated soil has been ineffective in winter season or a cold condition, because the number and activity of micro-organisms falls down in low temperature conditions. This study developed a new bioremediation technique for oil-contaminated soil clean-up by using a versatile foam. The goal of the study was to remove diesel-fuel in soils by just spraying surfactant foam on the top of the soil. The surfactant foam was specially designed for being stabilized for 12 hours and including low-temperature-microorganisms capable of degrading fuel. Versatile functions of the surfactant foam this study developed were to insulate the oil-contaminated soils from cold-weather conditions, prevent release of volatile organic carbons and dust from the soils, and provide micro-organisms and nutrients to the soils for biodegradation. The versatile surfactant foams were sprayed twice a day on the top of diesel contaminated soils present in a cold-chamber at 6 °C. Total petroleum hydrocarbons (TPH) were monitored as time elapsed. The experiment results showed that surfactant foam being placed on the soil kept the soil temperature 4 °C higher than ambient temperature and also clearly showed that TPH were significantly decreased by just spraying the versatile surfactant foam. This versatile foam spraying technique can be a promising sustainable remedial method minimizing energy and materials required for soil remediation. (This study was supported by the GAIA project (2012000550024) funded by Korea Environmental Industry & Technology Institute and Korean Ministry of Environment.) Keywords: biodegradation, soil, diesel fuel, surfactant foam

TU230

Ready biodegradability of poorly water-soluble substances: Evaluation of Bioavailability Improvement Methods (BIM) for Anthraquinone and Isodecyl neopentanoate in OECD 301 B test

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The biodegradation of chemicals as the bioremediation of polluted groundwater and toxic waste sites requires that bacteria have to be physically in close contact with the pollutant. Therefore, a low hydrosolubility may lead to underestimate the chemical's biodegradability. For example, a lipid compound with a less than 1 density floats at the air/water interface in the test vessel. In this configuration, the bioavailability of the chemical to microorganisms can be limited. Only few guidance documents have suggested technical adaptations to improve the bioavailability of poorly water-soluble chemicals. Their implementation could improve the biodegradation results. This aim of this study is to determine whether or not some Bioavailability Improvement Methods (BIM) can improve the biodegradability results of two poorly hydro-soluble chemicals, i.e. Anthraquinone (solid) and Isodecyl neopentanoate (liquid), in a Ready Biodegradation Test (RBT). The BIM performed are dispersion with ultrasound, emulsion with Humic acid, 2,2,4,4,6,8,8-heptamethylnonane (HMN), silicon oil or emulsifier addition, emulsifier/silicon oil dispersion, fixation on silica gel (with and without solvent), weighted on glass fiber filter and direct addition (as reference operating condition). Results of a first set of experiments performed with a screening RBT tool (Respicond VI) have already been published at SETAC Europe 2013 congress (poster TU175). This second communication compares these data with the new results obtained under the standard RBT OECD 301 B conditions. This study allows to conclude on the best BIM to evaluate the ready biodegradability of Anthraquinone and Isodecyl neopentanoate.

TU231

Structural similarity of organic aminomethylenephosphonates described by Tanimoto coefficient and its correlation with binding behaviour to a hydroxyapatite substrate

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Numerous organic phosphonate complexing agents are marketed in EU for a wide range of industrial purposes, or are described in academic literature. An important substance group is the aminomethylenephosphonates, which have two or more $-CH_2PO_3H_2$ groups attached to amine nitrogen atoms. All these substances have applications involving complexation and adsorption to solid surfaces, to present deposition of unwanted scales. Binding behaviour to calcium-based mineral substrates is of high importance in the context of risk assessment, and the understanding of environmental fate. A range of different structures exist, and similarity between the substances was investigated. A new way of understanding the complexation and adsorption was developed; this is a topic that eludes many normal approaches to QSAR. The capability of the substances to bind to hydroxyapatite reported in a key published reference source (based on *in vitro* data). Similarity was evaluated based on fingerprints, defined by the ring structures formed when different combinations of the ligand sub-structures bind to a metal ion (calcium). Similarity index scores relating to specific pairs of structures were found to be in the range 0.0 (dissimilar) to 1.0 (identical), indicating that while these substances share many properties in common, significant differences are to be expected at the level of interaction with calcium in a mineral matrix. The scores correlated with binding strength to hydroxyapatite, and also are relevant to published soil and sediment adsorption coefficients.

TU232

Effects of activated carbon amendment on Lumbriculus variegatus in PCB contaminated sediment: responses in freshly amended and aged systems

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Carbon amendments, such as activated carbon (AC), has strong sorption capacity towards hydrophobic organic chemicals (HOCs). It has therefore been studied as a potential stabilization method for contaminated sediments. Results received from various studies have been supporting the high efficiency of AC amendments in HOC sorption. Recently focus has been directed also to the possible adverse effects of AC on organisms. Although some adverse effects on benthic organisms have been observed, the desired effects of AC have been indicated to outshine the possible negative effects. Still, the subject lacks clarifying research data, which this study is aiming to improve. The objectives of our study were to investigate the responses of sediment dwelling oligochaete worm *Lumbriculus variegatus* in PCB contaminated sediments amended with coal based AC (ø 63μm-200μm). Both, the AC efficiency in reducing PCB bioavailability in sediments and ecological end points such as feeding, reproduction and growth were studied. In addition, the effect of aging of the sediment-AC system was investigated. The results showed that AC decreased PCB bioaccumulation. However, a clear AC dose related response in the studied ecological parameters was observed. Further, the adverse effects were evident still after 36 months of aging procedure. The AC dose required to trigger the response was dependent on the sediment. Results from the aging experiments will give indications of the possible long term effects of carbon amendments and may be useful when remediation measures are designed. Our results emphasize the importance of site-specific characters in remedial planning. Local ecosystems and possible adverse effects on organisms needs to be considered and the suitability of the carbon amendments on remedial purposes should be evaluated individually on each site in question.

TU233

The iso chemical structure on the ecotoxicity of ionic liquids

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Although ionic liquids can lessen the risk of air pollution due to their insignificant vapor pressure, they do have measurable solubility in water. The water is thus the most likely medium through which ILs will be released into the environment. Their physico-chemical and biological properties can be tailored by a judicious cation/anion combination, and allows them to be tailored as *quasi* specific fluids for one particular application. As a result of this diversity of combinations, ILs are often referred to as “designer solvents”. In fact, there have been attempts at understanding their biological impact in terms of the alkyl chain length, the anion, the cation core and the aromatic nature on their (eco)toxicity. However, despite the large number of papers reporting their “designer solvent” character, the impact of some specific structural alterations in the ILs behavior is still scarcely studied. In this context, the impact of structural isomerism on the ILs’ ecotoxicity using four distinct cations is here properly investigated. For that purpose, several Microtox® test assays were performed. The results suggest that it is possible to manipulate the biological impact by the incorporation of branched chains and that their influence on the ecotoxicity is dependent of the IL’ aromatic/non-aromatic nature.

Advancements in life cycle impact assessment and footprint method development (P)

TU234

Assessment of natural resource depletion – a surprising diversity: A quantitative comparison of impact models

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Natural resource depletion and material efficiency have become topics of great interest within resent years with many nations and supranational organizations developing strategies to mitigate political and natural limits to material consumption of our society. Within Life Cycle Assessment (LCA) natural resource depletion is a difficult and questioned topic: A wide range of impact assessment methods have been proposed in the literature, but little consensus has been reached on relevant safeguard objects or on the conceptual understanding of the environmental mechanism. In this study we make a quantitative comparison of four Life Cycle Impact Assessment (LCIA) methods on resource depletion through i) direct correlation of characterization factors and ii) correlation of characterized life cycle impact scores based on impact assessment of selected product systems. The LCIA methods included are CML 2001, Eco-indicator 99, IMPACT 2002+ and ReCiPe 2008. The study shows that CML has a very low correlation with all other methods comparing CFs (R^2: 0.21-0.46) while CML shows high correlation with IMPACT 2002+ and ReCiPe 2008 when comparing characterized impact scores. (R^2: 0.75-0.86). The opposite is the case when comparing Eco-indicator 99 with IMPACT 2002+ which have a strong correlation regarding CFs (R^2: 0.75) and a significantly lower correlation regarding the impact scores (R^2: 0.33). These deviations are caused by the energy resources in the assessment as they only constitute a minority of the CFs (4-5 resources) but have a decisive influence on the characterized impact scores of the product systems. The study also shows large differences in the correlation for the characterized impact scores between the individual methods. This is also an effect of the assessment of primarily the energy resources and differences in the resource depletion models of the different LCIA methods. The choice of LCIA method is shown to be crucial for the outcome of the study due to the differences in the depletion models. This emphasizes the need for building consensus on how to tackle resource depletion in LCA regarding the Area of Protection and the associated Environmental Mechanism.

TU235

How does the choice of ILCD’s recommended practice for characterization modelling change the assessment of environmental impacts in LCA of products?

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The European Commission has launched a recommended set of characterization methods for application in life cycle impact assessment (LCIA). However, it is not known yet whether the choice of the recommended practice, referred to as the ILCD, over existing methodologies matter for interpretation of LCA results. Here, we compare the ILCD with two of the most frequently used LCIA methodologies, IMPACT 2002+ and ReCiPe 2008, focusing on characterization at midpoint, by applying them on a case study comparing four window design options. First, to see whether the choice of ILCD matters for identification of product with the lowest environmental burden, ranking of the four window options was done for each impact category within each of the three methodologies. Next, impact scores calculated using each of the three methodologies were converted into common metrics for each impact category to see whether the choice of ILCD matters for total impact scores. Results show that apart from toxic impacts on human health and ecosystems, all three methodologies consistently identify the same window option as having the lowest and the highest total environmental impact. This is mainly because production of heat dominates the total impacts and there is large difference in demand for heat between the compared options. Yet, there were significant differences in impact scores for some of the impact categories after conversion to common metrics: above 3 orders of magnitude for impacts from ionizing radiation on human health and impacts from land use on natural environment; between 1 and 3 orders of magnitude for metal depletion and for toxicity-related impact categories; and within 1 order of magnitude for the remaining impact categories. These differences are caused by the differences in underlying characterization models and/or substance coverage, depending on the impact category. In summary, we showed that different LCIA methods, including

the ILCD, are likely to point to the same conclusion with respect to identifying the product with the lowest environmental burden, if one process is driving environmental impacts and there is large difference in demand for output from that process between the compared options. Nevertheless, the choice of ILCD' matters the most for assessment of impacts from ionizing radiation, land use, resource depletion (minerals), and all toxicity-related impact categories, where differences between ILCD and alternative methodologies are large. //

TU236

Reckoning of Operational and Environmental Benchmarks within 180 Wastewater Treatment Plants in Spain

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Wastewater treatment plants (WWTPs) are complex non-linear facilities whose operation is dependent on large perturbations in flow and influent composition, among other factors. Nevertheless, WWTPs must be operated continuously in the most efficient way in order to accomplish strict regulations for treated water discharge. The European Water Framework Directive (Directive 2000/60/CE) emphasized the importance of policy design and implementation for an efficient management of water resources within the European Union. The present study, within the framework of the Aquaenvec project (LIFE10 ENV/ES/520), proposes the combined use of Life Cycle Assessment (LCA) and Data Envelopment Analysis (DEA) to assess the operational benchmarking, eco-efficiency and environmental performance of the WWTPs under study. The methodology is divided in five main steps: 1-Life Cycle Inventory (LCI) for each individual WWTP, selected as the decision-making unit (DMU). 2- LCA for each DMU. 3- DEA from the LCI of first step. 4- LCA for the target DMUs. 5- Interpretation, eco-efficiency verification. From a DEA perspective, each WWTP represents a DMU. The minimum sample size needed to carry out the analysis will depend on the number of inputs and outputs chosen (Cooper et al. 2007). In this case, the inputs considered were two: Energy consumption (kWh) and Chemical consumption (kg). Regarding the outputs, two categories are distinguished: desired outputs (for which the method tries to maximize the results) and undesired outputs (for which the method minimizes the results). Treated water (m^3), BOD_5 removal (%) and Total Nitrogen removal (%) were considered as good outputs, while Sludge generation (kg) was a bad output. The sample used for the research after a data quality screening considered the LCIs of 180 different WWTPs. The ultimate purpose of the study is to give a clear picture of the eco-efficiency of WWTPs in Spain, and how this goal can be correlated with variables such as scale factor, climatic region or treatment technology.

TU237

The use of biomasses in the construction sector: the case study of hemp-based building materials

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Nowadays, there is a general consensus about the fact that the environmental impacts generated by the building sector are quite severe; nonetheless, this is a human activity which is considered indispensable for the future development; the house is perceived by most persons as a primary need and certainly a positive social and economic indicator. For sure, the sector is a major consumer of intermediate products and related services; moreover, the poor energy performance of buildings and the large use of non-renewable resources in the production and transport of construction materials may have a very detrimental effect on the environmental sustainability. Therefore, a careful choice of construction materials can very much help reduce global environmental impacts; so, a larger use of building materials of natural origin (e.g. wood, hemp, kenaf, cork, clay and lime) could be very beneficial to save non-renewable resources. In addition, most natural materials have thermal and hygrometric performances comparable to those of their synthetic counterparts. So, good thermal building insulation as well as high indoor air quality and comfort could be also guaranteed by adopting new paradigms in constructions. However, being natural is not sufficient to guarantee that a material or a product is more environmentally sustainable than a synthetic one. The main goal of this work is to assess the environmental sustainability of an emerging class of building materials of natural origin incorporating large amounts of biomasses: hemp-derived building materials; Indeed, hemp is a crop that in recent years is finding wide acceptance in building industry due to the fact that it requires no fertilizer or large amounts of water, has low energy consumption during cultivation, grows very quickly and has countless applications in the realization of buildings materials such as hempcrete or hemp-based bricks. In particular, hempcrete and hemp-based bricks have been analyzed on a cradle-to-gate perspective. The study follows assessment procedures and guidelines of international (ISO 14040-10044, EN 15804) and UK (PAS 2050) standards. A critical analysis on how to account for biomasses in the impact assessment of these products has been conducted, especially in the light of the recent European directive 2013/179/EU “on the use of common methods to measure and communicate the life cycle environmental performance of products and organizations”.

TU238

Using Life cycle assessments for the integration of environmental aspects in a sustainable controlling tool within the system of water supply and wastewater disposal

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Water supply is an indispensable prerequisite of society as well as of sustainable development. However, several risks as to the diverse dimensions of sustainable development have to be tackled for future water supply and wastewater disposal systems. Management of water systems requires to identify these risks and derive strategies, often by deciding between conflicting goals. In order to provide tools to stakeholders in water management to identify and manage future risks, a risk management system is envisaged which shall be combined with other management systems like environmental management or occupational health and safety. This risk management system will consider economic, ecological and social aspects which will be expressed by quantitative or qualitative indicators. As a first step of research, 16 categories of risk indicators have been defined which are influenced by external present as well as prospective causes just as climate change, demographic change, amendments to an act or changes in social structures. Life Cycle Assessment is the methological basis for quantitative assessment of environmental indicators. As a first step of research, the categories of risk indicators which have been defined will be analysed as to those aspects that are assessed by existing methodologies of impact assessment. Synchronous to this the management systems which are operated within the companies of the industry partners will be analysed to identify which indicators are already used and which inventory data are already collected. To keep additional work and expense of the industry partners to a minimum these indicators and data should be used for environmental impact assessment. The most interesting fact of the whole project is to combine the results of an impact assessment with those of other social and economic assessment methods to form a sustainable controlling tool which facilitates the industry partners to balance their investigations and to have a science-based argumentation for their internal and external communication especially facing politicians.

TU239

Regional Emission Account in Life Cycle Assessment of Agricultural System

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Up to date, life cycle assessment (LCA) was applied to many products, service and systems to evaluate their environmental impacts. Also there have been many developments on impact assessment methodologies and life cycle inventory database for LCA. Especially LCA for agriculture product and system is required regional data and characteristics, because soil and weather conditions in each region or country are different. However, the regional information and data is not well developed and connected with LCA. To better understanding of agriculture system and their life cycle environmental impacts, the regionalized emission information should be developed and considered in LCA. So, the research deals with integration of regional emission information to LCA in the example of agriculture system. For a case study, an industrial city of in Kazakhstan was chosen. Based on this industrial area, the regional emissions information such as Ag, As, Cr, Zn, Ba and Sb were developed by using sampling of black poplar leaves. Black poplar species is very well known as an effective indicator of pollution in an urban area that is why it was considered for the research. Analytical proceedings of samples were conducted by the instrumental neutron activation analysis with use of the nuclear research reactor of Tomsk Polytechnic University in Russia. The information on these emissions was converted to the mg/m^2 unites and maps of the distribution of these substances within the territory of the city was made. In the ReCiPe impact assessment method, three characterization factors and categories (human toxicity, terrestrial ecotoxicity, and freshwater ecotoxicity) which are related with developed regional emissions from our study were considered. After selecting 4 hypothetic sites which is agriculture area, we compared the characterization results between with/without regional emission information. Our results show that the characterization results, which are considered regional emission information, had much higher environmental impacts. Therefore, the regional emission information should be considered for LCA in agriculture products and system as well as constructing regional emission information.

TU240

Parameterized LCA models for geothermal energy systems: from detailed to simplified models by applying a Global Sensitivity Analysis

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COURTADE, BRGM / Direction des Géoressources Georesources Division; A. Beylot,
Environmental impacts of geothermal systems have been analysed with a life cycle perspective through a number of significant studies. However, several literature reviews have shown a large variability of environmental impacts and more specifically greenhouse gases ranging from 4 to 740 g $CO_{2\ eq}$/kWh and from 3 to 380 g $CO_{2\ eq}$/kWh. Such a large range of LCA results somehow discredits LCA as a relevant tool for policy makers. We propose to analyze and understand such variability by building relevant parameterized models based on key parameters explaining most of the variability. Our parameterized models are built upon inventory data related to the Bouillante geothermal power plant in the French Caribbean's as well as on published Life Cycle Inventories. This research aims at delivering two types of models: a general LCA reference model designed by LCA experts and a simplified model easy to use intended to neophyte LCA users. The simplified model is based on the reference model and allows users to obtain the main environmental information. Both models are designed under a parameterized pattern to allow their use for a large sample of different technical and scenario configurations. From the reference LCA parameterized model, we generate the environmental impacts profile representative of the potential configurations for the studied energy pathway, by taking into account the different variability sources (technological, geographical, methodological…). The Bouillante geothermal power plant life cycle inventory was used to define the reference model and to identify relevant parameters such as the depth of wells, the transport, the quantity of materials and the energy supply to produce materials like steel, concrete or gravel.

TU241
Analysis of Adequacy of LCA Comparison of types of walls on the ILCD methodology. Goal step to Inventory step.
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In an initiative of European Commission of Sustainable Consumption and Production and Sustainable Plan of Action on Industrial Policy (COM (2008)397) for the standardization of the methodology for the application of the study of life cycle assessment (LCA), the Manual of International reference Life Cycle Data System (ILCD) was developed and was adopted in Brazil as a reference for the Brazilian Program of Life Cycle Assessment (PBACV). This article aims to determine whether the assessment of the life cycle presented by an association of ceramic industries (AIC) in Brazil complies with the requirements of LCA methodology established by the ILCD handbook. The data presented in the technical report issued by expert consultants were analyzed, considering the steps of the goal, scope definition and inventory lifecycle, in order to use the data collected in this report for the Brazilian database of ACV. This was done by analyzing the report with the application of a questionnaire developed according to the steps required by the ILCD handbook. From each mandatory step described in the manual was elaborated a question whose answer indicates whether the requirement has been met or not. Then the requirements, completed items and items not completed, were systematized in a summary table. To comply with the methodology all requirements must be met. The questionnaire and the table were results developed to facilitate the process of analysis of LCA reports according to the methodology of ILCD, building support for the preparation of a proposal for a procedure for validating reports of ACV in the setting of PBACV. The completion of the verification report submitted by the AIC is that the responses of some requirements are incomplete and it is necessary to complete them properly for the report to fit the ILCD methodology. Among the requirements unfulfilled mention: methodological limitations were not identified, nor the solution adopted for them, the study was not rated among the situations A, B or C described by ILCD, and therefore does not point to attributional or consequential modeling, the steps for modeling the life cycle inventory (LCI) are not clear, etc. The noncompliance of requirements may impair the use of the AIC report data for the inventory of the ceramic block by PBACV, since the lack of information on the LCA may mask some results and compromise the comparison between products with the same functional unit.

TU242
Improving resources use and depletion assessment phase during LCA
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The notion of resources availability is complex since it covers different aspects: physical, economical, geopolitical, scarcity, which can be taken into account to assess the short and long term availability of resources. Therefore, different methodologies to support political and industrial choices aiming at securing resources availability for future generations have been developed, including criticality indicators. In that scope, various LCA related indicators have been designed in order to meet different needs in term of resources assessment. Yet, most of the LCA practitioners rely only on one of these indicators during their LCA studies. The SCORE LCA association, including leading industry players (EDF, GDF SUEZ, Renault, Saint-Gobain, Total, Veolia) and the French Environmental Protection Agency (ADEME), contracted BIO Intelligence Service to make an informed interpretation of the different approaches to quantify the impacts related to resource use in LCA, identify their strengths and weaknesses, and recommend improved practices. After a mapping of a selection of LCA indicators, a transversal analysis allowed to compare them, notably in terms of objectives and resources covered. Then, a detailed analysis presented the principles and foundations of the indicators, the calculation methods of characterisation factors, and inherent limits to each indicator, including data gaps. Three case studies were carried out to highlight and illustrate the differences between indicators and revealed that the use of only one indicator appears to be insufficient. Some main lessons were learned and will be elaborated during the presentation: The resources coverage taken into account by the indicators is variable and should be considered when choosing indicators. Regarding indicators covering both mineral and fossil resources, fossil resources generally come up as the main contributors. However, depending on the indicators and resources analysed, very variable contribution profiles can be observed. Depending on the resource indicator used, LCA conclusions can be divergent. In particular, when comparing different systems, the choice of the indicator / couple of indicators selected to assess resource use and depletion may influence the relative environmental performance of the compared systems. A final procedure is proposed to assess in a better way resources use and/or depletion within LCA, together with new research options to improve the existing indicators.

TU243
Regionalization of LCA using GIS: Environmental assessment of a coastal territory at a local scale.
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Currently, local authorities lack the methodologies necessary to assess environmental impacts at a territorial scale. Life Cycle Assessment (LCA) is a method that can assess environmental impacts in a life cycle perspective (from the cradle to the grave). LCA is widely applied to products or services; however, it is rarely used to assess the environmental burdens of a territory. LCA produces results at a global scale, but because environmental impacts can vary spatially, it seems necessary to take into account variability in impacts at a local scale. Although LCA is a promising tool to assess territories at a local scale (Loiseau et al., 2012), a methodology that include coupling between LCA and Geographical Information Systems (GIS) needs additional development. This work focuses on assessing local environmental impacts of a territory by coupling GIS with LCA. Several studies have previously examined GIS-based regionalized LCA (Geyer et al., 2010; Mutel et al., 2011; Dufossé et al., 2013); however, none have focused on assessing environmental impacts of a territory - including different resources and emissions from both agriculture and industry - at a local scale. A methodology will be developed by coupling LCA with GIS, where GIS will be used to regionalize the life cycle inventory (LCI) and life cycle impact assessment (LCIA) steps. Material transfers in environmental compartments (air, soil, water) will be determined using existing simulation models (e.g. CASIMOD'N, developed by INRA). This work focuses on coastal territories, and more specifically on two case studies in France: the *Lieue de Grève*, a territory polluted by green algae, and the *Baie du Mont Saint Michel*. Results will include testing different levels of data aggregation and performing uncertainty analyses.

TU244
Water deprivation at the sub-river basin scale at midpoint and endpoint level in LCA
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Physical water deprivation at the midpoint level is currently assessed in water-related LCIA methods using indicators that represent water scarcity at the river basin scale. Although these indicators have brought a great improvement to assess water impacts for conventional product LCA, significant challenges still remain to improve their accuracy and relevance. This is particularly the case for LCA practitioners who study foreground systems where water is a main issue (e.g., irrigated land area or water provision in big cities): they need to assess water resource options as well as downstream cascade effects for humans and environment at a more appropriate scale than the entire river basin. A new method for assessing water deprivation at the sub-river basin scale in LCA has been developed in order to integrate these downstream cascade effects. This method includes the fact that water consumption in a sub-river basin (SRB) deprives water in each downstream sub-river basins. The proposed characterization factor for water deprivation (CF_{WD}) of a SRB is the weighted sum of each downstream SRBs water scarcity (defined with the consumption-to-availability CTA ratio). Different SRB weighting parameters are proposed such as land area, volume contained within the rivers and human population. CF_{WD} has been initially defined at the midpoint level using water scarcity indices. Nevertheless, it appears that water scarcity has not yet been established as a relevant element of the endpoint indicators leading to ecosystems quality. Thus, the proposed approach also aims to apply and compare

the downstream cascade effect at the sub-river basin scale to existing endpoint indicators such as freshwater fish species damages due to water deprivation. Calculations of SRB scale CF_{WD} are done for two contrasted river basins: Seine and Guadalquivir. Sub-basins CTA and endpoint indicators are derived from literature data. Sub river basins delineation comes from a high-resolution digital elevation model. Results show similar tendencies at the midpoint and endpoint level: CF_{WD} at the upstream positions of a river basin are the highest since they deprive water for a large share of the river basin. In both river basins and with both methods, the difference between the minimum and maximum values is greater than one order of magnitude. This research shows the applicability and the interest of downstream cascade effects at the sub-river basin scale for both midpoint and endpoint approaches.

TU245
A novel multi-scale integrated framework for life cycle impact assessment of ecosystem services
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The evaluation of ecosystem services (ES) in LCA has focused so far on a few 'provisional' services (e.g., fossil fuels, wood, water, minerals,…), neglecting to assess the relevant contribution of (or the severe impact to) 'regulating' and 'supporting' services (e.g., nutrient cycles, erosion, pests and diseases, pollination,…). Despite novel characterization factors (CFs) for LCIA of ES have been proposed at the midpoint level, which are based on the harmonization between land use inventories and impact scores, the end-point LCIA characterization of natural resources and ecosystem health still neglects a significant number of ES. Moreover, the actual damage to ecosystem functionality and its relation to ES supply and pressure remain undefined. Interestingly, recent researches demonstrate the feasibility of using integrated earth system dynamic modelling perspective to retrieve time- and scenario-dependent CFs that consider the complex inter-linkages between natural processes delivering ES. Based on these studies, we intend advancing the state-of-the-art in LCIA of ES. Accordingly, we present a novel assessment methodology to be established within a project funded by the National Research Fund – FNR Luxembourg (VALUES: VALUing Ecosystem Services for environmental assessment). VALUES aims at defining a new biophysical-economic characterization method for LCIA of biodiversity and ecosystem services. Based on the marginal contributions analysis' perspective, VALUES enables both a quantitative and qualitative evaluation of the economic and environmental trade-offs and synergies due to the exploitation of ES at different scales (from micro/product- to meso- and macro-system scale). This approach can allow assessing (in the short- to long-term) the contribution of ES in supporting human and ecosystem welfare, by generating a number of CFs for LCIA under different future socio-economic scenarios (in marginal price values). CFs are retrieved from an improved version of the existing MIMES (a multi-scale Earth system model), optimized by using new modules of GIS and Input-Output datasets along with an integrated dynamic modelling system. The general framework of VALUES and its tasks are illustrated here with some preliminary results generated using a non-refined version of MIMES.

TU246
Approach for Assessment of Land Use Impacts by Agricultural Intensification: The Case of Ukraine
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The target of the EU directive on renewable energies (RED) is an energetic share of 10 % for renewable energies in the transport sector for all members until the year 2020. This target will mainly be reached by the use of biofuels largely produced from agricultural biomass. In order to provide additional biomass the following measures may be applied: yield increase, increase of agrarian area by land use change (LUC), reuse of abandoned / degraded land and the use of not area-related biomass resp. use of organic waste. Currently, LUC is discussed as countercurrent to effects like carbon stocks and greenhouse gas (GHG) mitigation. Priority is given to the use of degraded or abandoned land and yield increase. These measures need fertilizer, water and technical equipment. This is why a comprehensive assessment of possible impacts is crucial for future management strategies of land as well as biomass provision for Europe. According to several studies on global and European bioenergy potentials in various countries, a considerable potential is assumed in Eastern Europe. Particularly Ukraine with app. 3-4 M ha of unused agricultural land has a large potential to cover the future EU biomass demand by increasing its agricultural yields as well as by reusing abandoned and degraded agricultural land. Ukraine is considered as a promising area for future bioenergy production due to very favorable climatic conditions, fertile soil, good access to water resources and access to domestic markets. Nevertheless, an increase of agricultural production may cause, besides GHG-Emissions, negative effects on ecosystem services. The impact assessment of land use in life cycle impact assessment requires the modeling of several impact pathways covering ecosystem services. This poster presents an approach to determine the impact of land use by life cycle assessment in Ukraine as a case study region. According to the UNEP-SETAC guideline on global land use impact assessment on biodiversity and ecosystem services the application of the life cycle impact category "Ecosystem Service Damage Potential" will be applied on the region under study. The impact category includes Biotic Production, Climate Regulation, Freshwater Regulation, Erosion Regulation and Water Purification. The goal is an approach on an intermediate step to link the results with future land use impacts of biomass production in Ukraine and to evaluate the existing LCIA methodology on a regional scale.

TU247
A method for assessing green water flows: case study of Eucalyptus globulus forest in Portugal
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The on-going water demand in combination with the current climate change trends and land use changes could lead to irreversible disturbances on the terrestrial and aquatic ecosystems. The precipitation affects the terrestrial ecosystems, and in turn these ecosystems can also affect the precipitation (green water flow) that is recycled into the atmosphere due to land use changes. A reduction of the partitioning of the precipitation that is recycled into the atmosphere affects the heat flux that helps to control surface temperatures, bringing relevant implications for regional climate characteristics. This can affect the growth and evapotranspiration rates of vegetation. Despite the crucial relevance of green water use for the long-term sustaining of terrestrial ecosystem services, a Life Cycle Assessment (LCA) method focusing in the land use impacts on the green water flows remains a substantial gap. This study develops a LCA method to assess the land use impact on terrestrial green water flows. The Life Cycle Inventory considers the effects of the evapotranspiration recycling into the atmosphere due to land use changes, taking into consideration two scenarios of alternative reference land use: the quasi-natural forest and grasslands/shrublands. At Life Cycle Impact Assessment stage, spatial-specific characterisation factors are proposed. The applicability of the proposed method is illustrated by using the example of *Eucalyptus globulus* stands installed in several regions of Portugal. The impact results of the first rotation of *E.globulus* have showed that depending on the alternative reference land use, different environmental impacts are obtained. When considered the quasi-natural forest as alternative reference land use, the land use impact on green water flow (GWI) showed that *E. globulus* growth in Northeast and Central littoral regions cause no impact on the terrestrial ecosystem, which does not happen in the other regions analysed. Central region presented a GWI of 70.1 $m^3.ha^{-1}.yr^{-1}$, being the region with the highest potential to cause disturbances in the terrestrial ecosystem services. When the grasslands/subshrubs were considered as reference land use, no disturbances in terrestrial ecosystem were assessed for all the stands. The large range of GWI results shows that a clear understanding of what alternative reference land use should be considered in each specific land use system is of crucial relevance to obtain reliable impacts.

TU248
Water-stress characterisation factors for future-oriented LCA
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Steady-state water-stress based characterisation factors (CFs) for watersheds around the world are already available. CFs for impacts related to water use have proven to be highly spatially dependent. However, the influence of time in the obtained impact factors has not been analysed yet. To this end, this research aimed to calculate water-stress CFs for sub-watersheds in Spain for three temporal scenarios: current situation, short-term future (year 2015) and mid-term future (year 2030). Changing temporal trends in water use due to changing society and economy (ex., population, employment) and modification of regional freshwater resource availability due to already experienced and forecasted effects of climate change have been included in the calculation of the CFs. Statistical uncertainty information of the CFs for each time step is provided, taken into account that uncertainties grow with time horizon. CFs were calculated following the Water Stress Index (WSI) definition of a broadly applied water use impact characterisation method. The WSI was calculated on a yearly basis for 117 sub-watersheds covering Spain. Temporal tendency analysis of the computed CFs shows, in general, a relaxation of water stress in the short-term when compared to the current situation and afterthat a new increment. This is explained by the increase in water availability from now to the near future and then by both water use increment and water availability reduction. Large differences were detected in the comparison of the original WSI CFs at the watershed level for Spain and the WSIs calculated in this research. This may be due to the different time periods for which WSI are calculated and to the use of global maps and models used to figure out the original WSI versus regional data used here. The developed CFs are of usefulness to evaluate water use-related impacts of

present and future technologies with life cycle stages located in Spain. We have shown in this study that both temporal and spatial specification matters when assessing impacts of water use.

TU249

Land Use in LCIA: an absolute scale proposal for Biotic Production Potential.

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Environmental impacts caused by land occupation and transformation have been bypassed in many LCA studies due to soils' multifunctionality and the interconnectedness between the ecosystem services they provide. These inherent modelling complexities have traditionally forced LCA practitioners to content with a mere quantification of Land Use (LU), as surface area and duration (in m^2 or ha and years) appropriated by humans, without further analysis of the impact pathways derived from those land uses. Milà i Canals established the first comprehensive, basic framework for taking soil quality aspect into LCIA that reached acceptance among the LCA community. Through contributions from UNEP-SETAC's special task force on LU, great progress has ensued in developing further such LCIA. Building on the latest proposal by Koellner et al. and with the aim of bringing the Planetary Boundaries thinking into LCA, the present study proposes a single absolute scale for the midpoint impact category (MIC) of Biotic Production Potential (BPP). It is hypothesized that, for an ecosystem in equilibrium (where NPP equals decay), such an ecosystem has reached the maximum biotic throughput subject to site-specific conditions and no externally added inputs. The original ecosystem (or Potential Natural Vegetation) of a certain land gives then the maximum BPP with no additional, downstream or upstream, impacts. This Natural BPP is proposed as the maximum BPP in a hypothetical Absolute Scale for LCA's Land Use framework. It is argued that this maximum BPP is Nature's optimal solution through evolution-adaptation mechanisms, which provides the maximum matter throughput subject to the rest of environmental constraints (without further impacts). As a consequence, this scale rises a *Land Use Optimality Point* that suggests the existence of a limit regarding the maximization of divergent objectives with bioenergy. It will be attempted to model that beyond this point, and for the land available within a country, if the objective of Climate Change mitigation through bioenergy is further maximized, then the Fossil Fuel displacing objective will decrease, and vice versa.

TU250

Contribution to Arctic Climate Change from Countries in the Arctic Council

T.C. Schultz, SCS Global Services

This presenation will summarize research presenting the incremental contribution to Arctic climate change from emissions originating within the borders of the eight member countries and two largest observer states of the Arctic Council. The member countries include the United States, Canada, Russia, and the five Nordic countries, Denmark, Finland, Iceland, Norway, and Sweden. The two observer states included are China and India. The methodology used is from the life cycle impact assessment method for the impact category of Arctic Climate Change, defined in Section 4.2 of Annex A of the LEO-SCS-002 standard[1]. The method in the standard is the Absolute Regional Temperature Potential, a model developed by Shindell and Faluvegi that characterizes the Arctic surface temperature response to an emission.[2,3] For the member countries of the Arctic Council, the emissions data used are from the UNFCCC National Inventory Reports, and from the Arctic Monitoring and Assessment Programme 2011 report. For China and India, estimates of emissions of each substance were taken from several published sources. The characterization factor used to establish results is the Arctic Warming Factor (AWF), which is the ratio of the change in the Arctic surface temperature resulting from the emission of one kilogram of a substance to the change in Arctic surface temperature caused by the emission of one kilogram of CO_2. Within the countries which were considered, the five largest contributors to results, in order of their significance, are China, the United States, India, Russia, and Canada. The Nordic Countries collectively account for a much smaller contribution. Although it does not border the Arctic, China is the largest contributor to Arctic Climate Change. China, the United States, India, Russia, and Canada countries could respectively emit as much as 17.0, 12.4, 8.2, 5.5, and 1.2, Gigatons of CO_2e, calculated using the AWF-20. [1] Draft Standard for Type III Life-Cycle Impact Profile Declarations for Products, Services, and Systems, being developed under the open American National Standards Institute (ANSI) Process, administrated by the Leonardo Academy. [2] Shindell, D., and G. Faluvegi. *Climate response to regional radiative forcing during the twentieth century.* Nature Geoscience, Vol 2., April 2009, 294-300. [3] Shindell, D.T. Evaluation of the absolute regional temperature potential. Atmos. Chem. Phys., 12, 7955-7960, 2012.

TU251

Characterizing regionalized land use flows for Swiss forestry products and quantifying their impact on biodiversity

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To assess the impacts of land use on biodiversity, many methods were proposed in last decade but they lacked a regionalized approach and thus their application was limited and uncertainties high. Only in recent 1-2 years, a suite of methods have attempted to quantify biodiversity loss on a regional (biome, ecoregion or grid) level. The characterization factors are developed based on relative difference of species richness on different land use types compared to a regional reference. Switzerland imports several wood products from abroad and the production of these forestry products such as sawn wood, particleboard, plywood, furniture results in significant land use (occupation) or land use changes (transformation) in the countries of origin. Impact of these land use flows on the biodiversity of the region is often neglected or vaguely quantified when conducting their LCA because of the lack of reliable and operational impact assessment methods. This study first aims to characterize these land use flows from literature for typical Swiss forestry products (inventory data compilation). Impacts on biodiversity are then calculated using five different regionalized methodologies developed over last decade and results are compared (Impact assessment).

TU252

Quantifying variability in the carbon footprint of global wind power

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Every year more energy is produced by wind energy and in 2012 global wind capacity amounted to almost 283 GW approximately 3% of the world's energy production. Wind energy becomes increasingly important in replacing existing power plants as fossil fuels are predicted to become scarce and more expensive. With this increasing share of wind power in the electricity mix it becomes increasingly relevant to know the carbon footprint (kg CO_2eq/kWh) of wind power as accurately as possible. Many different studies have been undertaken to quantify the carbon footprint of wind power, with mixed results. Differences occur because of variability in wind turbine specific characteristics (e.g. nominal power, turbine height) and in location specific characteristics (e.g. transport distance, average wind speed). The combination of the location and turbine specific characteristics determines the amount of electricity that can be produced from that wind turbine and is therefore also a major determining factor for the carbon footprint. In this study we have quantified the variability in carbon footprints of wind turbines around the world. We have used the WEPP database (World Electric Power Plant) with turbine specific characteristics and locations for over 10000 different wind turbines and parks throughout the world. By coupling the location of these wind turbines to global wind maps, location specific wind profiles and load factors could be determined.

TU253

A method for calculating the "grey water footprint" using the life cycle approach

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The concept of 'water footprint' introduced by Hoekstra (2003) and subsequently elaborated by Hoekstra and Chapagain (2008) provides a framework to analyze the link between human consumption and the appropriation of the globe's freshwater. The water footprint of a product is defined as the total volume of freshwater that is required to produce a good considering its life cycle. The bluewater footprint refers to the volume of surface and groundwater directly consumed in the production processes; the greenwater footprint refers to the rainwater consumed, whereas the greywater footprint of a product refers to the volume of freshwater that is required to assimilate the load of pollutants based on existing ambient water quality standards. Although the concept and the method have become widely applied, few works have focused on the calculation of the grey component of the indicator. The major concern has been on the fertilizers and agrochemicals in agricultural stages of production. Additionally, no study in the literature has successfully incorporated the life cycle approach in water footprint methods. Recently, a compendium of methods has been included in the new version of SimaPro 8, dealing with various impacts of water related issues such as human health and water scarcity. In this study, a method for calculating the grey water footprint is proposed. The objective is to provide the potential impact related to pollutant emissions to the water compartment using a life cycle approach expressed in terms of volume of water required to dilute the emissions to accepted and defined water quality standards (according to the Brazilian law). In this way, it is possible to calculate the grey water footprint considering the agricultural and industrial stages of production, accounting for the indirect impacts associated with all inputs used. The SimaPro 7.3.3 software and Ecoinvent 2.2 were used as auxiliary tools. The production of butanol from sugarcane in biorefineries in Brazil was investigated. Results show that the method is able to provide good comparative information for the environmental viability analysis of technological scenarios.

TU254

TOX-TRAIN: the user-friendly toolbox for human and ecotoxicity assessment in LCA

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It is estimated that 10 to 20 thousand different chemicals are regularly used in the life cycle of products, many of which can potentially have harmful effects on humans or ecosystems. Industries are more and more interested in understanding the toxicity of their products constituents and are willing to act on their supply chain to reduce the amount of dangerous substances emitted through all stages, from manufacturing and processing to end-of-life. The four-year EU-funded TOX-TRAIN[1] project aims at creating a user-friendly toolbox based on the scientific consensus model USEtox® and promoting this model, in order to assess human toxicological and ecotoxicological impacts related to emissions over the life-cycle of technologies. Developed under the supervision of the Life Cycle Initiative lead by the UNEP-SETAC, USEtox® assesses such impacts for numerous chemical substances[2]. The main output of TOX-TRAIN will be a toolbox that combines the official USEtox® model with additional assessment tools in a user-friendly interface along with a full documentation TOX-TRAIN has already achieved several of its objectives. A thorough literature review of existing chemical inventory models was made, which led to the development of the sewage emission model SewageLCI[3]. This model is based on a set of chemical and national input parameters capable of quantifying the fraction of a chemical emitted to sewage systems ending up being subsequently released to the individual USEtox® emission compartments. Exposure models for household, workers, and direct exposure were developed and will constitute additional modules of the TOX-TRAIN toolbox. Characterization factors for persistent bioaccumulating chemicals, persistent active surface chemical compounds, pesticides and biocides have been developed and their uncertainty quantified[4]. All these developments will be tested and evaluated in case studies. Finally, several courses and workshops have been organized at different venues to inform and train on USEtox® and its application within the LCA framework. Outputs and development from TOX-TRAIN will all become publically available and will be evaluated for future integration into USEtox®.

TU255

AIIDA: THE ONLINE DATABASE FOR SHARING AND COMPUTING ECOTOXICITY DATA IN THE CONTEXT OF REACH

J. Payet, Cycleco; O. Hugonnot, ToolsEnv; E. Maillard, Cycleco

New environmental regulations such as REACH or Product Environmental Footprinting require having a vision of the environmental profile of commercial substances or products. In spite of huge efforts that were done since 30 years to run ecotoxicity tests for thousands of substances, it is still complex and time consuming for scientists to get a clear perception of the potential risk or impact of a chemical substance. It is the purpose of AiiDA (Aquatic Impact Indicator DAtabase) to provide a quick and clear overview of all ecotoxicity data for more than 10 000 substances and a detailed calculation of the main risk and impact indicators commonly used in the regulation. The AiiDA platform groups together the most important ecotoxicity databases and provides more than 500 000 ecotoxicological bioessays on more than 3600 species and belonging to 30 phyla. The data update is provided every 6 month and take into account all the available tests of ECHA (REACH compatible) and US-EPA Ecotox database. This global database is used to automatically calculate different ecotoxicological metrics and their uncertainties according to the Technical Guidance Document (*TGD*) from the European Union. The calculation of the Species Sensitivity and Phyla Sensitivity Distributions are provided to the user automatically. The species tested in AiiDA are linked to the ITIS database (*Integrated Taxonomic Information System*) and can be classified according to their geographical origin. The calculated risk or impact metrics (PNEC, HC_5, HC_{50}) are available in the interactive platform that provides the results, the traceability of all the calculation steps and the region specificity of the endpoints. This traceability doesn't exist in the usual tools addressing Risk or Life Cycle Assessmet and ensure the consistency of the aquatic indicators. These experimental data are primarily variables that can be used as input models such as USEtox, from which are calculated potential ecotoxicological impacts in life cycle impact assessment, environmental footprinting and ecological risk assessment. The AiiDA platform allows comparing the toxicity of these different molecules and all data are fully traceable, this transparency is of main importance for Ecodesign by allowing the user to analyse each endpoint. In the future, this community platform database can be completed by each user who can suggest improvements or add data.

TU256

COMPARING METHOD AND DATA AVAILABILITY FOR CALCULATION OF CHEMICAL FOOTPRINT

J. Payet, Cycleco; O. Hugonnot, ToolsEnv; E. Maillard, Cycleco

The increase of chemicals use and applications has led to a decrease of the global ecological health of surface waters. While the importance of these ecotoxicological impacts has been observed in numerous aquatic environments, it is necessary to manage several strategies in order to reduce the impact of chemicals on freshwater ecosystem. Calculating the chemical footprint of substances enable ranking f chemicals in order to identify those which have the highest burden in environment, and possibly prioritize the use of chemicals with the lowest burden. Nevertheless, the chemical footprint needs to be based on reliable and transparent ecotoxicity data, in a consensual method. While the method is currently under development, available data and corresponding limitation need to be checked. It is the purpose of this work to check available databases and to compare existing data with the needs of each method in order to estimate how many chemicals can be covered by the chemical footprint. In addition, among presented methods, the presentation gives a specific focus on the applicability of the Usetox ™ model for calculating environmental footprint and the link with current regulation such as REACH. The data required for each method of calculation are then describes and the maximum coverage is therefore assessed. Base on existing availability of ecotoxicity and degradability data, it appears that the chemical footprint could be calculated for several thousands of substances, but the variability of the results can change with the chemical of the results leading to a limited efficiency for substances comparison.

TU257

Towards a more robust fate modelling of metals' long-term emissions in an LCIA context

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Heavy metal long-term emissions from landfills are problematic in terms of LCIA due to the long time frame they occur coupled with their relatively low concentrations. The time integration principle of LCA leads to an (artificial) overestimation of their impacts since the emissions are treated as one large pulse emission, which leads many practitioners to, alternatively, cut off a high proportion of potential future leaching of metals. In an attempt to address this issue, the fate factor FF, used in the calculation of characterisation factors for the toxicity of metals, is examined in terms of its future development. The range of all possible combinations of globally measured values for the parameters most influential to fate, namely K_d, soil organic carbon and soil erosion rate, provides a spectre within which the FF is possible to develop in the future. By using this spectre of FFs, future scenarios can be constructed, namely best- and worst-case scenarios for the development of fate and ultimately characterisation factors (if the bioavailability, accessibility and effect factors remain constant). The produced series of characterisation factors for each scenario can be matched with time frames of inventoried time-dependent emissions of cationic metals from landfills, found in literature. Therefore time-dependent toxic impacts of metals emissions to soils can be estimated, the integration of which over time gives an overall best- or worst-case toxic impact of metals leaching from a landfill. The proposed framework avoids the integration of emissions, which overestimates the resulting toxic impacts, and does not ignore any future emissions (both of which constitute the current common practice in LCA). The impacts of long-term metal emissions under this framework are more accurate and could contribute to a more informed decision-making.

TU258

Including the introduction of exotic species in life cycle impact assessment: the case of inland shipping

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While the ecological impact of anthropogenically introduced exotic species is considered a major threat for biodiversity and ecosystems functioning, it is generally not accounted for in the environmental life cycle assessment (LCA) of products. In this article, we propose a framework that includes exotic species introduction in an LCA context. We derived characterization factors for exotic fish species introduction related to the transport of goods across the Rhine-Main-Danube canal. These characterization factors are expressed as the potentially disappeared fraction (PDF) of native freshwater fish species in the rivers Rhine and Danube integrated over space and time per amount of goods transported (PDF·m3·yr·kg−1). Furthermore, we quantified the relative importance of exotic fish species introduction compared to other anthropogenic stressors in the freshwater environment (i.e., eutrophication, ecotoxicity, greenhouse gases, and water consumption) for transport of goods through the Rhine-Main-Danube waterway. We found that the introduction of exotic fish species contributed to 70−85% of the total freshwater ecosystem impact, depending on the distance that

goods were transported. Our analysis showed that it is relevant and feasible to include the introduction of exotic species in an LCA framework. The proposed framework can be further extended by including the impacts of other exotic species groups, types of water bodies and pathways for introduction.

TU259

Pursuing an ecological component for the Effect Factor in LCIA methods

N. Cosme, Technical University of Denmark DTU / DTUMAN QSA; A. Bjorn, Technical University of Denmark / Department of Management Engineering; R.K. Rosenbaum, National Research Institute of Science and Technology for Environment and Agriculture Irstea / UMR ITAP

Life Cycle Assessment quantifies the environmental impacts from emissions and resources consumption of human activities. Uncertainty in modelling natural processes and ecological regulation challenges the prediction of effects from further pressures. Ecosystems' health and adaptation capacity may have also been altered by past impacts. Model frameworks are usually built on stability, linearity of causality and expectation of a safe return to stable states if the stressor is minimised. However, the command-and-control paradigm has resulted in the erosion of natural resources and species diversity. Ecosystem-related impacts are traditionally benchmarked by potential loss of biological diversity as Potentially Disappeared Fraction of species (PDF) integrated over area and time, building on the biological sensitivity of species in each receiving ecosystem. For consistency among Life Cycle Impact Assessment (LCIA) methods midpoint indicators are shown in Potentially Affected Fraction of species (PAF), which implicitly suggests reversibility to previous stable states. Currently applied conversion factors from midpoint to endpoint (species loss, as PDF) range from 10 (NOEC-based), 2 (chronic EC_{50}-based) or 1 (assuming that continuous stress affects reproduction rate), but these are all based on biological/physiological responses and do not add a true ecological component to the impact. Such factor simply changes the HC_{50} by 1 or 0.3 log units. A stressor with equal intensity in two differently disturbed ecosystems (close or distant to a threshold) and sharing similar biological communities should not result in, necessarily, the same impact potential. We suggest the introduction of an ecological term in the Effect Factor of the characterisation modelling for ecosystem quality-related indicators in LCIA. An application to a marine eutrophication indicator will be presented to show how impacts from nitrogen emissions vary with the individual receiving ecosystems' health, by defining proxies for ecosystem's state and resilience. These, express the pressure on the system and its propensity for regime shifting. Ultimately, the ecosystem's capacity to tolerate the pressure, to adapt to the stress and minimise its effects should complement the biological response. In our view, adding an ecosystem-based approach to the damage estimation can positively contribute to the environmental relevance and spatial differentiation of the results.

TU260

Large Marine Ecosystems and coastal water archetypes implemented in LCIA methods for marine eutrophication and metals ecotoxicity

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The marine eutrophication (MEu) and marine ecotoxicity (MEc) indicators in Life Cycle Impact Assessment (LCIA) respectively express the eutrophying impact of nitrogen (N) and the toxic impact of metals emissions to the marine environment. Characterisation Factors (CF) are calculated to translate the emissions into impact potentials. For consistency in the characterisation modelling across impact categories, the same modelling framework was applied including Fate Factors of N or metals (FF), habitat Exposure Fate (XF) in MEu or Bioavailability Factor of metals (BF) in MEc, and Factors for the Effect on biota (EF). In both impact categories there is a need for spatial differentiation according to the receiving ecosystems, and the parameterisation of the characterisation models requires the adoption of suitable spatial units out of the global receiving coastal marine ecosystem. The Large Marine Ecosystems (LME) biogeographical classification system identifies 64 spatial units of coastal marine waters and it was adopted for both MEu and MEc. The applicability of 13 alternative zonation systems was compared before choosing the LME classification. The hydraulic residence time (RT) of the receiving LMEs expressing the system's flushing through local hydrodynamics is required for the parameterisation of the FF term to estimate the loss of N or metals from the LME through advection. The RT was found in literature for 36% of the LMEs, whereas 4 archetypes were built for the remaining, for which no data was found (47%) or to settle high variability of found sources (17%). The 4 archetypes were defined by the exposure to currents and regional marine circulation, depth and profile of the continental shelf, and stratification. Archetype 1 (high dynamics and exposure) with estimated RT=3 months, Archetype 2 (medium dynamics and exposure) with RT=2 yr, Archetype 3 (low dynamics) with RT=25 yr, and Archetype 4 (very low dynamics, embayed, often stratified) with RT=90 yr. It is assumed that the system dynamics is determining the RT of both N and metals in the photic zone in each LME. The LME classification system was chosen for its data availability, modelling feasibility, and adequacy of size and number of spatial units considering the needs of LCIA. The application of the archetypical RTs was useful for the parameterisation of the fate models. The spatial differentiation of the resulting CFs was found essential to increase the discriminatory power of the models.

TU261

Assessing chemical footprint of Europe to support chemical policies for products

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In the last few years, environmental footprint (EF) concept has obtained an increasing interest by both the scientific and political communities. Nowadays, EC-JRC is developing a life-cycle based methodology for assessing EF (EC-JRC and DG ENV, 2012) based on ILCD recommendation (EC-JRC, 2011). Amongst the other footprints, the chemical footprint evaluation aims at assessing at which extent actual emission of chemicals harm the ecosystems above their capability to recover (the so-called carrying capacity of the system) and could be used to support integrated policy for chemicals. In a recent (2009) paper in Nature, Rokstrom et al highlighted that for some environmental problem (such as climate change or global freshwater use) thresholds for a safe space for humanity were already set. This means that it is possible to evaluate the relative performance of human activities against this threshold. For other specific issues, such as chemical pollution, the thresholds setting is uppermost relevant even if very critical to be defined. Actually, the potential harm caused by a particular amount of a chemical released to the environment depends on a number of interrelated factors, including the properties of the chemical and the medium to which they are released. Due to the complexity of this interaction, especially for ecosystem, a specific multidisciplinary effort has to be made to perform integrated assessment of chemical pressure. Chemical footprint evaluation should support possible product policy options towards a "PAF (potentially affected fraction) zero" target, namely supporting prioritization of chemicals to be substituted/banned to achieve relevant reduction in ecotoxicological effect. This should be coupled with the assessment of link/synergies with Reach regulation, not only in terms of data availability for physical- chemicals properties and toxicological data but also in terms of tracking usage of substance both in the manufacturing/production, use and final fate steps.

TU262

The French Ecotoxicity Footprint project is using the Usetox model for assessing impacts of products on freshwater ecosystems

J. Payet, E. Maillard, Cycleco; O. Hugonnot, ToolsEnv; C. Roussel, Cycleco

The environmental impact assessment of chemicals and mass market products requires multiplying emissions of chemicals during the whole life cycle of a product by characterisation factors of substances, for various impact categories. These characterisation factors are calculated with models developed for different impact categories, within the life cycle assessment methodology. For ecotoxicity, the USEtox™ model has been developed under the UNEP-SETAC Life Cycle Initiative by a team of researchers. This model is the state-of-the-art for the ecotoxicological impact assessment of substances. In France, it has been selected by the french government for the ecotoxicological footprint of different product categories. In this context, Cycleco was committed by the French Environmental Agency (ADEME) to assess the possibility of using usetox for calculating the ecotoxicological footprint of products. The main outcomes of this project can be summarized as follows: the model can be applied for products and chemicals footprint assessment. Nevertheless, in its original form, USEtox™ is clearly designed as a research model and is not easily applicable by industries, that need a pragmatic and reliable tool. For a regulatory purpose such as environmental footprinting, the model as it stands is missing a clear governance, a complete documentation, a full transparency and is also impeded by the limited number of data. This three year project allowed to apply USEtox™ for various kinds of substances and products (cosmetics, detergents, pesticides), and will ended in March 2014. It is providing solutions for all the limits identified and allow industries to easily use the model for conception or regulatory purposes. This three-year project demonstrates the feasibility of using USEtox™ for calculating the ecotoxicological footprint of chemicals and products with limited adaptation.

TU263

New characterisation approaches within the 4th generation ecological scarcity method: abiotic resources and nuclear waste

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The ecological scarcity method is a life cycle impact assessment method based on the distance to target approach, including classification, characterisation and normalisation. During the past two years the Swiss eco-factors based on the ecological scarcity method have been updated for the third time. Like the previous

versions, the 4th generation eco-factors Switzerland are based on the same distance to target formula. The new version covers some additional impact categories, and some revised characterisation approaches related to existing impact categories. The new elements are: radionuclides emitted to air and freshwater bodies, persistant organic pollutants emitted to freshwater bodies, abiotic resource depletion (in addition to water and gravel/sand), biome-specific land use covering land use in all biomes world-wide, noise from transportation (road, rail, air), and a new characterisation of nuclear wastes. The presentation will focus on two new elements: Abiotic resource depletion and nuclear waste assessment. Abiotic resources are characterised using the abiotic depletion potential concept published by CML in 2001 and updated information about global reserves and global annual production. Additionally, it is recommended to apply the impact factors on the share of a resource lost by final disposal and/or dissipation. Hence, borrowing resource use and consumptive resource use are distinguished and only the latter is included in the impact assessment of abiotic resource use. Hence, abiotic resources are assessed in a similar way like water use, where consumptive and borrowing water use is distinguished too. Up to now, nuclear wastes were assessed based on a rather vague political target. In the new version the different nuclear wastes (e.g., low level and high level wastes) are characterised based on their radiotoxicity, an internationally accepted measure of the radiation potential of the wastes. Because the radiotoxicity of 1 ton of high level waste is up to 20'000 time higher compared to 1 ton of low level waste, the high level wastes are far more important than the ten times larger volumes of low level wastes. The presentation will additionally show results of selected case studies applying the 4th generation eco-factors Switzerland.

TU264

Dynamic and geographic extensions of LCIA for incorporation with an integrated assessment model

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In this study, we explored methodology to give LIME targeted to current status of Japan, to time dynamics and geographical extension to global. In correspondence with the impact category of LIME, we dealt global warming, urban air pollution, land use and its change, ozone layer depletion, mining and disposal of mineral resources. Evaluation factors of LIME are incorporated to our integrated assessment model (IAM) whose time horizon and geographical coverage is up to 2100 and global 10 regions, respectively. Damage coefficient factors of LIME associated with resource consumption deal with three end points; namely, resources as resource depletion, biodiversity by land use change, and net primary productivity (NPP) by land use and land-use change. The latter two are treated in this investigation. Damage coefficients provided by LIME (whose units have kg-NPP/kg-metal, EINES/kg-metal) is composed from two factors, namely "land use area per weight of resources consumed" and "land use area per damage amount". These factors are multiplied to the produced mineral resource and damaged amount per land use change endogenously obtained from the integrated assessment model. The above described damage factors of LIME extended for dynamics and geography are incorporated to the IAM to make simulation for the future dynamics of global environmental change. Figure 1 shows breakdowns of environmental external costs when they are internalized into macroeconomic relations, and that the largest share is land-use and its change, followed by global warming. All the other is minor. Share of impact on land-use and its change still continued larger but shows declined tendency along the path. We assumed that the effect of reducing the environmental impacts of forest conservation is relatively large, which suppresses impacts by development. On the contrary, global warming shares about 15% at present, grows up to about 30% in 2100. This is because due to the population growth in the future, CO2 emissions from deforestation and energy consumption in developing countries will increase significantly.

TU265

How to use the available knowledge in LCIA to help understand relative importance and relevance in trade-offs and help making an informed decision

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The life cycle impact assessment method recommended by the European Commission for the Product Environmental Footprint (PEF) and the Organisation Environmental Footprint (OEF), based on ILCD, is a list of 15 indicators at midpoint. The challenge with this recommended list is when conflicting answers are given depending on the indicator assessed. The objective of this work is to present how to use damage-oriented knowledge to increase analysis capacity, allowing to identify wich indicators are dominating "absolute" impacts and therefore should be considered in priodity when making a choice between two products. Information based on endpoint and/or damage oriented methods such as IMPACT 2002+, ReCiPe, or IMPACT World+ [http://www.impactworldplus.org] are used to generate conversion factors to go from midpoints recommended by ILCD to damage indicators. Those factors are used to understand better the trade-offs between products, as for example between rape oil and soya oil. When using information available in the damage oriented litterature applied to the case study of rape oil and soya oil, one sees that impact categories contributing to damage to human health are driven by particulate matter, followed by toxicity, but with ozone depletion, ionizing radition and photochemical orone formation being all three significantly smaller. When analyzing impact categories contributing to damage to ecosystem quality, one sees that rape oil has overall slightly more impacts than soya oil. So overall, one sees that rape oil has either equal or more impacts then soya oil (but not less impacts). Therefore, with current information, using damage oriented information, one can better discriminate between two products showing trade-offs in terms of environmental impacts. This presentation will show the thinking process and how using damage oriented information can help increase application/usefulness of the impact assessment method recommended by the European Commission for Product Environmental Footprint (PEF) and the Organisation Environmental Footprint (OEF).

TU266

"Base IMPACTS ®": setting up a database to support the French footprint programme on mass market products

O. Rethore, ADEME

In the context of the French laws aiming at an environmental labelling on consumer goods, the ADEME has been mandated to set up a national LCI/LCIA database: the Base IMPACTS ® (http://www.base-impacts.ademe.fr/). In terms of format, ADEME's database is based on the JRC's ILCD Dataset format. Each dataset is imported as an aggregated life cycle inventory (LCI) dataset and characterized through the methods recommended by the JRC, allowing the LCIA indicators to be released to the public. In terms of content, three modes have been set up to feed the database in terms of LCI datasets: purchase (mode 1), development (mode 2), and contribution (mode 3). Mode 1 relies on the "purchase" of existing processes through framework contracts with PE International, Cycleco, Ecoinvent and Quantis. 14 subsequent contracts have already been signed, covering intermediate systems such as electricity, transportation, plastics, etc. For sectors with lacks of data, the ADEME sets up collaborative projects to develop LCI datasets. A Unit Process version of each dataset is also released, allowing more thorough eco-design approaches. Choices made to model the systems are gathered and detailed through public methodological reports. Two projects are now over on agriculture (Agri-BALYSE ®, [1]) and pulp and paper production, one is ongoing on food processing (ACYVIA ®, [2]), and new ones remain to be launched: on chemicals production, on wastewater treatment plant (with the aim to allocate the impacts per chemicals), on plastics or textile recycling, etc. The third mode will allow third-parties to propose their own data. To come along with this work on the datasets, around 1.500 aquatic ecotoxicity characterization factors have been developed following the USEtox ® model through a partnership between ADEME and Cycleco, to be implemented into the Base IMPACTS ®. The presentation will show the latest developments and the main features of the Base IMPACTS ®, in terms of content, associated comitology and management rules. It will try to highlight both its strengths and weaknesses, and to propose answers to some of the questions that raise as challenges for such database projects: how to deal with consistency within the whole database? how to guarantee reproducibility within Mode 2 projects? how can one integrate such a work on intermediate systems with the development of B2C Product Category Rules? how do the constraints of public consultation impact to such projects? etc. Links and references [1] http://www2.ademe.fr/servlet/KBaseShow?sort=-1&cid=96&m=3&catid=25508. [2] http://iterg.com/-etudes-et-recherches-14-304

TU267

Carbon Footprint of Printing Industry in China and Data Quality Analysis

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Responding to global climate change, carbon footprint has gained tremendous popularity. In the paper, we took printing as a service and guided the work of emission reduction effectively based on the true carbon emission level in this industry. Meanwhile, we established the carbon footprint model of book and plastic flexible packaging printing service, including system boundary, unit function, the principle of data delete and distribute, and data collection. The data collection scheme was completed by the field research of four typical book printing companies and two typical plastic flexible packaging printing companies, primary data include generation, energy consumption, material consumption, waste and traffic, and second data are mainly carbon emission factors. On the basis of the book printing service industrial characteristic and data collection availability, this paper selected the function unit as 1 plate, 1 sheet and 1sheet according to the three printing stages, respectively. The results show that CTP plate is the mainly carbon

emission source in the pro-printing stage, differences between printing technologies have a significant impact on the printing carbon footprint, circulation process play an important role in the post-print stage. In the plastic flexible packaging printing carbon footprint, the unit function was selected as 1roller, $1m^2$ and $1 m^2$, respectively. According to the results, selecting the right roller provider, chemistry and compound technology, in turn, are the key factors to the pro-printing, printing and post-printing stages carbon footprint. And the dry compound carbon footprints are often higher than the extrusion compound. This paper combined the uncertainty and sensitivity two factors to estimate the data quality during the carbon footprint. The primary and secondary data uncertainties were evaluated by the DQI-Monte Carlo method, and we obtained uncertainty base on the error transfer principle. Simultaneously, this paper gained the main data in the assessment process with the sensitivity. The data with bigger uncertainties and higher sensitivities would be the key data and the key point of enhancing the result credibility and refining the data collection in the carbon footprint.

TU268
The effect of land use changes on greenhouse gas emissions in the wine sector: a case study for the Ribeiro appellation (NW Spain)
P. Villanueva, University of Santiago de Compostela / Chemil Engieering; I. Vázquez-Rowe, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; M. Otero, R. Blanco, University of Santiago de Compostela / Department of Geography; M.T. Moreira, G. Feijoo, University of Santiago de Compostela / Chemical Engineering
Land use changes (LUCs) have shown to trigger relevant effects on environmental impacts in a wide range of studies developed to date. In fact, LUCs have demonstrated to account for a considerable amount of the greenhouse gas (GHG) emissions generated in the agricultural sector. While GHG emissions linked to LUCs at a regional or national level have been widely studied in the literature, the effect that these changes may have at a micro-scale remains unexplored. The viticulture sector accounts for an important proportion of agricultural land in Spain. In fact, Spain is the country with most land destined to grape production worldwide, above countries like Italy or France. However, in many wine-producing areas in Spain important LUCs have occurred in recent years due to changes in the wine sector, including a higher focus on producing quality wine rather than priming productivity, or the gradual disappearance of numerous small-holdings in favor of medium and large scale wineries. The current study delves into the LUCs occurring in an emblematic wine appellation in NW Spain, *Ribeiro*, which has suffered important socioeconomic and demographic changes during recent decades. For instance, the area destined to vines in the valley has decreased from approximately 4450 ha in 1990 to 3000 ha in 2010, despite the observed increase in wine exports and in wine quality. Afforestation in the highlands of the valley and, to a lesser extent, agricultural intensification and increase of vineyard surface in areas of lower altitude, are the two main phenomena observed in this period. Therefore, the specific objectives of this study were to analyze from a Life Cycle Assessment perspective the annual dynamic changes in GHG emissions in the period that ranges from 1990 and 2010 in this wine-growing area, with special focus on the effect that LUCs have had on the final emissions. For this, a geographic information system (GIS) was used to evaluate the LUCs. Thereafter, guidelines from the IPCC were used to assess the carbon retention and/or emission from the soil, as well as regional factors and parameters to improve the quality of final results. The final results, which are currently being processed, intend to provide support to policy makers in the viticulture sector to understand current trends in terms of carbon retention and emission in the appellation.

TU269
Characterisation factors for aquatic biota damage due to suspended sediments in water column
p.S. quinteiro, A.C. Dias, University of Aveiro / Department of Environment and Planning CESAM; A. Araujo, Univeristy of Aveiro / Department of Mechanical Engineering; b. ridoutt, CSIRO; L. Arroja, University of Aveiro
Sediments in freshwater streams may come from several sources: soil erosion, mining and construction activities. On average the European soil erosion is in the order of 8.0 $t.ha^{-1}.yr^{-1}$. Suspended sediments (SS) that reach the freshwater streams can be a source of potential environmental harm. The term SS describe fine particulate matter with a diameter of less than 62 μm. The high levels of SS can be significant stressors to the biodiversity of aquatic organisms. This aspect is not presently considered in LCA and is a substantial gap considering the scale of the issue. High concentrations of suspended sediment can damage the gills and small appendages of aquatic macro-invertebrates. Both periphyton and macrophytes require light penetration throughout the water column for photosynthesis purposes. The increase of the turbidity of the water column due to SS reduces light availability, which reduce the photosynthesis rates, leading to lethal effects on these primary producers. This study develops a fate and effect method to calculate endpoint characterisation factors addressing the direct effects of SS on the macro-invertebrates and periphyton/macrophytes organisms. The applicability of the proposed method is illustrated by deriving characterisation factors for aquatic biotic damages due to SS from topsoil erosion by water. The soil erosion by water has been characterised as one of the most upsetting problems in rivers. The European average soil erosion by water is about 2.8 $t.ha^{-1}.yr^{-1}$, being possible that during episodic storms, this rate can easily achieve 20 $t.ha^{-1}.yr^{-1}$. A set of 22 river sections distributed around Europe were studied. The fate factors reflect the environmental residence time of SS in each studied river section per unit of water volume. These factors are equal for the all the studied aquatic organisms and were calculated based on gross soil erosion data, SS delivered to rivers and sediment yield. The effect factors quantify the potentially disappeared fraction of aquatic organisms. These factors were determined based on a dose concentration-duration response model. The on-site monitoring of SS concentrations is crucial to improve the aquatic environment-sensitivity to the SS stressor, and to calculate characterisation factors at a wider range of river sections. **Keywords:**fate and effect modelling, aquatic biota, ecological damage, suspended sediments

TU270
Limits of the USEtox methodology for environmental labelling of rinse-off cosmetic products
J. Lharidon, LOREAL / Life Sciences Direction; P. Martz, LOréal Research Innovation; J. CHENEBLE, LOREAL; j. campion, LOréal Research Innovation
Several environmental labelling projects for consumer products are under study at national (*e.g.* France and USA) and international (*e.g.* EU) levels. They plan to take into account their impact on aquatic ecosystems. The USEtox model, developed for Life Cycle Assessment (LCA) within the framework of the joint project SETAC-UNEP LCA Initiative, is recommended (France) or is expected to be recommended (USA and EU) for calculating the environmental impact of certain consumer products, especially shampoos and shower gels. As things stand, USEtox has three disadvantages that call into question its ecological relevance and consistency with regulatory approaches concerning the assessment of the environmental impact associated with substances. (1) Assessment is based on average toxicity for all species taken together and not on the toxicity observed in the most sensitive species (2) A chemical substance may have a negative environmental impact according to USEtox, yet be considered of no foreseeable risk to the environment according to the criteria of the REACH regulation. (3) The results are often discordant with those found using the Critical Dilution Volume (CDV) method, the latter being necessary to obtain the European Ecolabel for soaps, shampoos and conditioners. The CDV method would appear to be a suitable alternative to USEtox for environmental labelling of rinse-off cosmetic products like shampoos and shower gels. The present study compares the results obtained with the USEtox and CDV methods applied to 33 shampoo constituents and 367 shampoos of the L'Oréal Group. Our current arguments in favor of the CDV method for environmental labelling of rinse-off cosmetic products will be detailed.

TU271
Spatial and temporal variation of health damage factors for PM2.5 and ozone on a continent level
L. Tang; T. Nagashima, National Institute for Environmental Studies; K. Hasegawa, ChudenCTI CoLtd; T. Ohara, National Insittute for Environmental Studies; K. Sudo, Nagoya University; N. Itsubo, Tokyo City University
Secondary aerosols and ozone produced by air pollutant emitted in one location can move on an intercontinental or hemispheric scale. Especially concentration of ozone has a significant temporal variation due to meteorological conditions. The aim of the research is to estimate human health damage factors of $PM_{2.5}$ and ozone on 10 regions of the world by using one global chemical transport model. Here the regionality results of $PM_{2.5}$ are reported, the result of ozone with seasonality will be presented on the conference. The damage factors express the change in disability adjusted life years (DALYs) of the world due to change in emissions of black carbon and organic carbon (BCOC), nitrogen oxides (NO_x) and sulfur dioxide (SO_2). The 10 regions' damage factors were calculated by three steps. Firstly, after we divide the whole world into 10 regions, the concentration change of the world caused by a change in emission of a substance from one region was estimated with a global scale model MIROC-ESM-CHEM for $PM_{2.5}$. Secondly, DALY change on the world due to a change in concentration was estimated by using population data and epidemiological concentration-response functions (CRF) of mortality and morbidity. Finally, above calculations were done for all 10 regions. Based on the method above, damage factors of SO_2, NO_x and OCBC for 10 regions were estimated. The difference of DFs can be up to 1 order of magnitude among 10 regions in all 3 target substances. One of the main parameters determining the DFs is the population density, however the variation of transport of $PM_{2.5}$ on continent level between emitted regions also have a significant influence. In Europe, Russia and Middle east regions, the values of damage occurred outside of emitted region estimated as 1/4, 1/4 and 1/3 of their DFs respectively. It is disclosed that the DFs will be underestimated when the transport of $PM_{2.5}$ to outside is not taken into account in those regions. For further research to quantify the differences of DFs among regions, it is important to consider the regional CRF and DALY per case.

TU272

Indoor exposure to emissions from wood products: Complementary views from life cycle assessment and risk assessment
A. Abhishek, ETH Zurich / Environmental Engineering; S. Hellweg, ETH Zurich / Institute of Environmental Engineering
Indoor wood products are known to emit volatile organic compounds (VOCs) into the air of buildings during their use phase. Chronic exposure to these offgasing emissions causes adverse human health effects and may lead to sick building syndrome. Indoor concentrations of these chemicals can significantly exceed indoor air quality goals particularly in low ventilation settings. However, existing life cycle assessment (LCA) studies of these products do not take into account the use phase health effects from indoor exposure. Such an omission is an important shortcoming, as it hinders a fair comparison with the environmental performance of competitive products and may result in underestimation of product's total environmental burden. In this study, from the published literature of last three decades, we identified which VOCs are emitted from most commonly used indoor wood products and compiled their emission factors into a database. The meta analyses showed that formaldehyde, acetaldehyde, acetone, pentanal, hexanal, α-pinene and β-pinene are the most frequently emitted VOCs. This compiled use phase inventory data is ready to be added to existing inventories of these products. which till now only document the emissions occurring during production and disposal phase of their life cycle. For the imapct assessment, indoor intake fractions were combined with USEtox effect factors to quantify human toxicity impacts from wood emissions. It was found that the use phase human toxicity impacts are in general an order of magnitude higher than those occuring during rest of the life cycle. For the risk assessment, concentrations of emitted VOCs were compared to their occupational threshold levels. Results complement those obtained using LCA and confirm the fact that wood based materials emit VOCs that may render the indoor environment unsafe from human health and comfort point of view, particularly in the initial months of installation. We conclude that the use phase which is generally neglected in the LCA of wooden products can actually be a hot spot in their life cycle.

Monitoring the efficiency of risk mitigation measure protecting the environment from pesticide exposure and effects (P)

TU273
Clarification analysis for bentazone findings in groundwater reservoirs in Germany
W. Koenig, Federal Environment Agency UBA; S. Karl, UDATA; A. Mueller, Federal Environment Agency / IV; C. Pickl, Federal Environment Agency Germany / Section IV Pesticides Fate and Behaviour Groundwater Risk Assessment; K. Thomas, Institute for Agroecology; M. Trapp, RLP AgroScience IfA / Institute for Agroecology
Referring to monitoring data from the federal states for the time period from 1996 to 2011, bentazone is identified as the active substance of plant protection products with the highest frequency of about 1% of nationwide findings in groundwater above the authorisation limit concentration in Germany. Based on a field clarification analysis, runoff and bank infiltration from field adjacent surface water bodies into groundwater, leaching in soil as well as vulnerable soil and hydrogeological conditions in limestone areas have been identified by the authorities as the main possible causes for bentazone findings. As a consequence, risk mitigation measures are remitted since several years: No application of herbicides with bentazone (a) >1000 g as/ha (b) on soils with organic carbon below 1%, (c) on sandy soils, (d) in potatoes, (e) before the 15th April, (f) field application only with covered buffer stripes of 5 m next to surface water bodies. A rank correlation analysis conducted with the 24 most sold herbicides indicates that the high frequency of the bentazone findings cannot be explained by the sale volumes of respective herbicides only, but most likely results from a combination of unfavourable substance properties for leaching, such as a high mobility in soil and a high persistence in water. A geostatistical analysis was conducted to investigate the spatial distribution of the findings, and to identify circumstances and conditions which allow bentazone to reach the groundwater. Finally, there is no clear evidence that the risk for groundwater contamination from the use of bentazone is restricted to areas with vulnerable soil conditions with regard to leaching in soil (low organic carbon content, very sandy soils). The results of the GIS analysis let rather assume that clayey and loamy soils with a potential to develop shrinking cracks and macropores are additionally vulnerable refererring to bentazone transport into groundwater. Taking into account hydrogeological data, the permeability of the geological underground may further aggravate the risk for groundwater. Based on the results of all available monitoring data analysis the efficacy of remitted risk mitigation to reduce bentazone findings in groundwater is highly questionable and currently under discussion in Germany. It is recommended to discuss options and/or limitations of groundwater risk mitigation measures during the renewal of the approval of bentazone under Regulation (EC) No 1107/2009.

TU274
Selection of worst-case scenarios for groundwater risk assessment using spatial data
B. Kind, WSC Scientific GmbH
For the registration of plant protection products in the EU a groundwater risk assessment must be performed to demonstrate the safe use. This is usually done using simulation models (i.e. FOCUS), but occasionally monitoring studies are required. Ideally, the monitoring sites as well as the modeling parameters are representative for most regions of the EU and show worst-case situations. One model to identify groundwater bodies which are more vulnerable than others is GeoPEARL. But so far it is only available for the Netherlands, although attempts were already made to use it EU-wide. The aim of this study was to investigate an alternative method to identify those worst-case regions in Europe, based on spatial data in a GIS. The leaching into groundwater of PPPs depends on the degradation and adsorption behavior of their active substances and metabolites. The degradation and adsorption behavior on the other hand, may be influenced by multiple factors like soil, crop and weather conditions. To distinguish the most sensitive factors, FOCUS-PEARL runs were conducted for fictional substances with different degradation and/or adsorption behaviors and for the four FOCUS-scenarios Chateaudun, Kremsmünster, Porto and Jokioinen. These scenarios were chosen as they have a wide range of soil texture, OM content, pH values, annual precipitation and annual mean temperatures. The four scenario soils were combined with the four climate scenarios to estimate the different effects of weather and soil properties. The PEC values were then analyzed using a multiple regression to quantitatively evaluate which scenario properties or which combination of these have the strongest influence on the 80th percentile PEC_{GW}. The Akaike information criterion (AIC) showed that the data were best described by model with parameters organic matter content, annual precipitation and/or annual mean temperature. The results from the regression were used to calculate a susceptibility index in GIS. This was done exemplarily for the Netherlands, in order to compare the results with predictions by GeoPEARL. The GeoPEARL runs were performed with the substances and application pattern used for the FOCUS-PEARL simulations. The areas of concern identified with both methods revealed largely similar results. While the aim of the presented method is not to substitute leaching models such as GeoPEARL, it offers an efficient alternative to identify potential areas of concern regarding groundwater assessment.

TU275
The use of monitoring data of Swiss surface water in the post-registration process
K. Knauer, Federal Office for Agriculture / Section Plant Protection Products
National monitoring programs to survey the environment and describe the current status of environmental compartments such as surface waters are widespread in Europe. These programs are performed on a regular basis and as a result tremendous data are available. In Switzerland, the monitoring of the surface waters are performed by the cantons. From 2005 to 2012, 345 000 samples have been taken and analyzed for various plant protection products. Samples were taken in small (< 2km2) and bigger surface waters such as the river Rhine. These measured environmental concentrations were compared to regulatory acceptable concentrations obtained during the authorization process and based on data provided by industry. The outcome of this survey and its implication for product management will be discussed.

TU276
Atrazine Ecological Monitoring Program (AEMP): Stewardship and Best Management Practices (BMPs) in Runoff-Vulnerable Watersheds of the U.S.
C. Truman, Syngenta Crop Protection Inc; M. White, S. Chen, T. Barlow, Syngenta Crop Protection LLC; D. Campbell, Syngenta; C. Harbourt, Waterborne Environmental; A. Lynn, L. Carver, Waterborne Environmental Inc
Certain watersheds across the U.S. are potentially susceptible to runoff and agrichemical transport, especially when runoff-producing rainfall events occur just after agrichemical application. We present an integrated stewardship program that builds on Syngenta's extensive atrazine monitoring database by coupling farmer education, outreach, and information exchange with adoptable, field- and landscape-scale BMPs to reduce runoff and atrazine losses in vulnerable watersheds monitored within the AEMP. Stewardship program components at the State and watershed levels will be presented and include: cooperating/communicating with State and local agencies and organizations; understanding and applying knowledge of watershed characteristics (soils, topography, hydrology, agricultural practices, erodibility); the science supporting atrazine efficacy and transport; and site-specific, field- and/or landscape-scale research supporting BMP effectiveness and adoption. This stepwise approach emphasizes a sustainable stewardship paradigm that improves soil and water management while improving overall water quality. Outcomes of the stewardship program include quantifying the effectiveness of individual and/or combined effects of stewardship activities and BMPs, reducing runoff and atrazine concentration and load trends exiting vulnerable watersheds, and economical and

effective weed control. Results will be site-specific and could be transferred and/or extrapolated to other watersheds potentially vulnerable to atrazine runoff.

TU277
The use of dye tracers in field-based regulatory studies to quantify pesticide spray drift
L. Fogg, ADAS UK Ltd / Cambridge Environmental Assessments; T. Pepper, Cambridge Environmental Assessments
Quantification of off-target spray drift deposits may be required to demonstrate mitigation effects of the application apparatus compared to reference systems, or to show the effect of buffer zones or in-field structures. Several field and laboratory methods exist for the measurement of spray drift deposition (drift fallout), either through direct measurement of the pesticide or the use of a tracer. Fluorescent dye tracers have been used extensively to validate claims of 'low drift' performance of agricultural and horticultural spraying systems for many years. Depending on the dye, high sensitivity can be achieved, making them a practical alternative for measuring spray drift in regulatory studies. Dye tracers allow for multiple/repeat applications to be made to the same area enabling a more precise dataset with reduced variability to be produced as well as comprehensive testing to be conducted under a range of climatic conditions. In addition, large numbers of field samples can be processed quickly and at relatively low cost. The resulting generic dataset can also be used to support multiple actives in the aquatic risk assessment process. However, there can be disadvantages associated with using dye tracers, particularly, instability of a dye during an experiment and poor extraction efficiency leading to low recovery rates. Interference from trace elements and other impurities present in the water used to prepare the spray solution and of that used for extracting residues as well as water temperature, can all have a marked effect on the accuracy of the fluorescence measurement. The approach detailed in this poster shows how some of the above mentioned pitfalls associated with the use of the dye tracer, brilliant sulpho flavine, were identified and overcome, and how this dye was successfully used as a surrogate compound for determining pesticide drift in two large regulatory field studies.

TU278
Quantifying the efficiency of vegetative buffers in removing pesticide contained in surface runoff - under a range of field and climatic conditions
T. Pepper, Cambridge Environmental Assessments; G. Hughes, N. Brettell, Cambridge Enviromental Assessments
FOCUS Landscape and Mitigation v2 (FOCUS, 2007) includes vegetated buffer strips as an option for reducing pesticides in surface runoff. However, default values for pesticide removal efficiency of buffer strips are not compound specific, are not FOCUS scenario specific, and may be too low to demonstrate compliance for specific compounds in all circumstances. Compound specific experimentally derived values for buffer strip removal efficiency may be useful to demonstrate compliance, especially if the field data generated is FOCUS relevant, and would compliment or be complimented by current modelling approaches, for example, VSFMod. The study described here suggests a step-wise approach to deriving representative test conditions and also the development of a replicated field plot test system where pesticide in "run-on" water calculated from the selected events was applied and measured across differing widths of buffer strip. Standardised tests were carried out in the UK with pesticides of differing physicochemical characteristics, and under a range of soil moisture conditions. Mechanisms responsible for in-buffer attenuation of pesticide contained in runoff were assessed, as well as the total mitigation provided by each combination of buffer width and field/climatic parameters tested.

TU279
Mitigation measures to reduce the runoff and erosion in experimental field in Poland according to recommended TOPPS-Prowadis manual – Best Management Practices
M. Bielasik-Rosinska, Institute of Environmental Protection NRI / Environmental Risk Assessment; D. Maciaszek, I. Kondzielski, Institute of Environmental Protection NRI
Surface water protection is one of the main tasks in sustainable use of pesticide developed by EU. In parallel, European Crop Protection Association started to promote water pollution reducing in TOPPS projects. Initially projects were focused on point source (2004) and now – on diffusive sources (TOPPS-Prowadis, started in 2011). The **Best Management Practices,** run-off manual, developed by TOPPS-Prowadis experts and partners (7 Member States, 8 institutes and universities and companies, PPP producers, ECPA.) is a base to choose the mitigation measures for runoff and erosion from fields and catchment. Different measures were proposed and implemented in audited experimental catchment in Żelazna near by Skierniewice, Poland: vegetative buffer zones (on the fields edge, in-field); retention structure (wet meadow); stone bunds in talweg to reduce water flow in concentrated runoff ground dams on the edge of field. The main task of them is to protect surface water from the pollution, mainly chemicals – plant protection products and fertilizers which are leached after rain. The second one is to keep water in the field. The positive effect of implemented mitigation measures was just observed during the project duration and will serve to protect surface water in the catchment. ***Special thanks to the runoff TOPPS-Prowadis team***: Folkert Bauer (BASF), Jeremy Dyson (Syngenta), Guy Le Henaff (Irstea), Volker Laabs (BASF), David Lembrich (Bayer CropScience), Julie Maillet Mezeray (Arvalis), Benoit Real (Arvalis), Manfred Roettele (BetterDecisions), Emilio Gonzales (Univ. Cordoba- ES), Aldo Ferrero (Univ. Turino-IT), Klaus Gehring (Landesanstalt für Landwirtschaft-DE),Marian Damsgaard (Danish Agr. Advisery Service, DAAS-DK), Ellen Pauwelyn (InAgro-BE).

TU280
Agri-environmental indicator for the risk of aquatic ecotoxicity
S. Spycher, Agroscope ChanginsWaedenswil Research Station ACW / Analytical Environmental Chemistry; L. de Baan, ETH Zurich IED / NSSI; O. Daniel, Agroscope ACW
Agri-environmental indicators are defined and calculated to monitor the effects of agriculture on the environment. Indicators are valuable tools to monitor if efforts to reduce risks posed by PPP on the environment are effective or not. For this purpose indicators on the of PPP-use and the concomitant risk for the environment are necessary. In Switzerland the collection of data on the PPP-use is integrated in a general monitoring of agri-environmental indicators: a network of more than 300 reference farms reports annually data on crops, PPP-use, fertiliser-use, ecological compensation areas, etc. The data are used to calculate different PPP-use indicators such as PPP-interventions or amount of active ingredients used per year for different crops. An agri-environmental indicator for the risk of aquatic ecotoxicity is calculated based on the model Synops. Ecotoxicity in this model considers acute and long-term endpoints for fish, daphnids, algae and macrophytes. Exposure assessment assumes a still standing or flowing water body and calculates concentrations of the combined input via drift (based on Rautmann-values), run-off (curve number method) and drainage (meta-modell based on MACRO). Precipitation, temperature, slope and soil type can be varied in the model. Synops calulates for each parcel acute and long-term exposure toxicity ratios (ETR). In the poster we present results on different possibilities of aggregating the ETR over time, PPP and active ingredients, organism groups, environmental parameters, parcels on a farm and crop types. The influence of the selected environmental conditions on the risk differs strongly between crop types. While the calculated risk varies very little for orchards, it is highly variable in the case of field crops. Ideas on the choice of representative parameters are discussed.

TU281
Implications of climate change on the sustainability goals in the use of plant protection products in Germany
K. Kuppe, Umweltbundesamt / Plant Protection Product; S. Huck, S. Pieper, German Federal Environment Agency UBA / Plant Protection Products; S. Matezki, Plant Protection Products
Climate change, mitigation measures and adaptation strategies in the energy and agricultural sectors will have serious impacts on the use of plant protection products (PPPs). Pest management strategies will be adjusted with respect to different agronomic parameters. Consequently, the ecological potential of the agricultural landscape to compensate the negative effects on biodiversity directly connected to pesticide use may be altered. As a result, the probability of land use conflicts (e.g. cultivation of energy crops on formerly unused or extensively used land) may increase. The objective of the research project is to identify potential conflicts of sustainability goals in the use of PPPs on the one hand and climate change mitigation and adaptation strategies on the other hand. For that purpose the current state of knowledge of direct and indirect impacts of climate change on agriculture will be outlined. Parameters such as species range, crop rotation, infestation pressure, patterns of exposition and spray sequence will be analyzed in order to derive the main factors relevant for scenario-based projections of the prospective pesticide use. The outcome will be a regionally differentiated projection of PPP use in representative agricultural regions in Germany. Based on these results, the implications of climate change and scheduled mitigation and adaptation measures on the sustainability goals in the use of PPPs (as laid down in Directive 2009/128/EC) will be critically examined. Potential synergies and conflicts with the intention to assure a sustainable use of pesticides will be identified in order to establish and evaluate practical recommendations on the optimization and harmonization of pest management and climate change mitigation/adaptation strategies. The poster will highlight the objectives of the project and provide an overview on the methodical approach.

Risk assessment of chemical mixtures: strategies, bottlenecks, and the steps ahead (P)

TU282
Development of the Hydrocarbon Block Method for Environmental Risk Assessment of Petroleum Products

M. Comber; A.D. Redman, Exxon Mobil Biomedical Sciences; T. Parkerton, M. Leon-Paumen, ExxonMobil Biomedical Sciences Inc; C.V. Eadsforth, Shell International; K. den Haan, CONCAWE / Petroleum Products Safety
This poster is number 1 of a series of 5 posters on the risk assessment of petroleum substances CONCAWE has been conducting a programme assessing the risks to man and the environment complying with the requirements of REACH. The products have been grouped according to previously agreed categories for classification, with consolidation based on composition and intended use. The approach adopted for assessing the environmental fate and effects of these categories is based on the Hydrocarbon Block Method (HBM) as described in the Technical Guidance supporting REACH. The approach that CONCAWE have adopted reflects the comprehensive nature of the single substance approaches normally used, but allows for the complex nature of petroleum products. The HBM has been used for all categories requiring an environmental risk assessment. The poster describes the basic approach and introduces the overall strategy: 1. Analyse substance composition & variability 2. Select HBs to describe product composition 3. Compile relevant physical-chemical & fate properties for HBs 4. Estimate environmental emissions of HBs throughout product lifecycle stages 5. Characterize fate factors & intake fractions of HBs 6. Determine environmental exposure to HBs 7. Assess environmental effects of HBs 8. Evaluate individual and aggregate risks of HBs

TU283
Analytical Characterization of Petroleum Products
C.V. Eadsforth, Shell International; M. Comber, Mike Comber Consulting; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; A.D. Redman, Exxon Mobil Biomedical Sciences; T. Parkerton, ExxonMobil Biomedical Sciences Inc; K. Den Haan, CONCAWE
This poster is number 2 of a series of 5 posters on the risk assessment of petroleum substances Detailed two-dimensional gas chromatography (GCxGC) compositional analysis has been carried out on petroleum products to support environmental and human health risk assessments of these substances under REACH. Following high performance liquid chromatography (HPLC) fractionation of saturated and aromatic components, GCxGC was used to generate quantitative data on >300 hydrocarbon groups and individual components. This information was used to characterize the distribution of mass among the different chemical classes and carbon number intervals. Structures, based on the CONCAWE library, were then used to define and populate the hydrocarbon blocks. The hydrocarbon blocks were subsequently employed in quantitative calculations (i.e. PETROTOX/PETRORISK, see posters 3 and 4 in this series) to conduct hazard and risk assessments for environmental and human exposure scenarios of the petroleum products. GCxGC data across the different categories are shown, demonstrating basic similarities within categories, the variability that exists between CAS numbers, and the limitations with the higher boiling point products.

TU284
Applications of PETROTOX in hazard assessment of petroleum substances
A.D. Redman, Exxon Mobil Biomedical Sciences; T. Parkerton, ExxonMobil Biomedical Sciences Inc; M. Comber; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; C.V. Eadsforth, Shell International; K. Den Haan, CONCAWE
PETROTOX relies on a library of 1500 representative structures as an extension of the CONCAWE hydrocarbon block method (HBM) to support risk and hazard assessment of complex petroleum substances. These structures are used to model the dissolution and toxicity of petroleum substances. Available analytical data are used to characterize distribution of mass among different chemical classes and carbon number (or boiling point) intervals. The representative structures are then mapped to these blocks and assigned mass weights based on the analytical characterization. The library includes structures from major chemical classes between 4 and 30+ carbon numbers, which represent most major petroleum substances (e.g., gasoline to gas oil to bitumen, etc). Environmental partitioning properties were estimated for each structure using SPARC and toxicity is modeled assuming concentration addition using the target lipid model. PETROTOX has been validated previously for prediction of acute effects and more recent applications include prediction of acute and chronic effects of a wider range of substances and categories. In combination with passive sampling methods, PETROTOX is used to streamline testing programs by identifying candidate test substances (e.g., toxic vs nontoxic) and test concentrations. PETROTOX combines the multi-component dissolution behavior of complex petroleum substances and provides a consistent basis for evaluating hazards across a wide range of petroleum substances.

TU285
PETRORISK – an Excel based tool for conducting environmental risk assessment of petroleum substances
M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; A.D. Redman, Exxon Mobil Biomedical Sciences; K. Den Haan, CONCAWE; T. Parkerton, ExxonMobil Biomedical Sciences Inc; M. Comber; C.V. Eadsforth, Shell International
This poster is number 4 of a series of 5 posters on the risk assessment of petroleum substances PETRORISK is a modeling framework used to evaluate the environmental risk of petroleum substances under typical use conditions. Petroleum substances are complex substances comprised of hundreds to thousands of individual constituents. The physicochemical, fate and effect properties of the individual constituents within a petroleum substance can vary over several orders of magnitude, which complicate the risk assessment. PETRORISK combines the risk assessment strategies used on single chemicals with the hydrocarbon block approach to modeling complex substances. Blocks are usually defined by available analytical chemistry, which characterizes substances in terms of mass fractions for discrete blocks based on chemical class and physicochemical properties (e.g., carbon number or boiling point). Emissions and predicted exposure concentrations (PEC) are modeled using mass-weighted representative constituents. Overall risk for environmental compartments at the regional and local level is evaluated by comparing the PECs for individual representative constituents to their predicted-no effect concentrations (PNEC) derived with the Target Lipid Model. Risks to human health from indirect environmental exposure may also be evaluated using the overall predicted human dose to a substance-specific Derived No Effect Level (DNEL).

TU286
Hydrocarbon Block Approach for Petroleum Products risk assessment - Summary and Conclusions, including further work
M. Comber; A.D. Redman, Exxon Mobil Biomedical Sciences; T. Parkerton, M. Leon-Paumen, ExxonMobil Biomedical Sciences Inc; C.V. Eadsforth, Shell International; K. Den Haan, CONCAWE
This poster is number 5 of a series of 5 posters on the risk assessment of petroleum substances CONCAWE has been conducting a programme assessing the risks of petroleum products to man and the environment to comply with the REACH legislation. The products have been grouped according to previously agreed categories for classification, with consolidation based on composition and intended use. The approach adopted for assessing the environmental fate and effects of these categories is based on the Hydrocarbon Block Method. This method has been used for all categories. Although all the Petroleum Products have been registered under REACH, in the process of conducting these risk assessments the results have been addressed and research needs for furthering the approach taken, identified and initiated. The poster will describe the outcome under REACH and the research now being undertaken to improve the assessment of the environmental fate and effects of petroleum products. Covering: ? Summary of the Petroleum Products for which risk assessments were conducted and key recommendations for further work ? Further work aimed at refining the Hydrocarbon Block Method and its constituent parts

TU287
Assessing the cost effective potential sequential extraction offers to industrial management of steel process by-products.
K. Rodgers, University of the West of Scotland; A. Hursthouse, S. Cuthbert, University of the West of Scotland / School of Science
In 2012 126 million metric tons of crude steel was produced worldwide with up to 400kg of solid waste created per tonne produced. There are potentially toxic elements (PTEs) present in the waste by–products, of particular interest in this research are Cr, Cu, Ni, Mn and Zn, which require robust management to minimise environmental contamination. Within the steel industry it is vital to be able to differentiate between chemical forms of elements as this can determine disposal routes and maximize efficiency e.g disposal of waste per tonne is typically £72 for hazardous and £2.50 for non-hazardous waste at current UK prices. Currently regulatory tests (WAC and TCLP) are designed to assess and dictate these disposal approaches, which is dependent on their chemical form or speciation. Successful speciation of solid samples for routine analysis has yet to be established for the steel making industry, however the material efficiency of the processes might be improved by better waste characterisation achieved through the application of sequential extraction (SE). Increased material use through improved process yield can be integrated with waste management allowing industrial symbiosis by working towards zero waste operations. SE methodology of PTE's can be used to operationally speciate the solid by–products into separate phases (including sulfides). This study reports on the development and application of a 7-step SE method to provide waste characterisation protocols, which evaluate the impact of waste origins and reproducibility on final waste classification. The main process by-products contain wide concentration range of PTE's. This is due to the semi-batch nature of the process, with varying degrees of recycled materials added during production, and consequently highly variable waste over time. The method has been developed and applied to a series of active steel plant waste samples. Initial data shows for Flue dusts, that PTE's of interest are found predominately in $FeOx_{am}$ and $FEOx_{cryst}$ phases whereas filtercake also has a high percentage of PTEs in the sulfide phases. The fractionation scheme was assessed to understand the impact of extraction solutions on solid phases, using a number of solid phase

techniques on sacrificial samples to confirm characterisation (XRD, FT-IR, Raman spectroscopy and SEM). Reproducibility was tested against total recovery compared to pseudo-total metal content using ICP-AES.

TU288

Ecotoxicological assessment of the environmental compatibility of construction products for outdoor applications

I. Heisterkamp, F. Flach, Hydrotox GmbH; U. Schoknecht, BAM Federal Institute for Materials Research and Testing; N. Bandow, Federal Institute for Materials Research and Testing / Contaminant Transfer and Environmental Technologies; M. Burkhardt, HSR Hochschule für Technik Rapperswil; C. Dietschweiler, University of Applied Sciences; M. Ratte, ToxRat Solutions GmbH; O. Ilvonen, German Federal Environment Agency; S. Gartiser, Hydrotox GmbH

The European Construction Products Directive allows Member States to establish comprehensive rules on the assessment of environmental impacts of construction products such as information on the release of substances and requirements on the use phase of construction products. Harmonized standards are currently developed under CEN / TC 351. For products producing complex leachates which require extensive analytical effort direct testing of the leachates in bioassays is a promising option. In total 20 construction products that are used outdoors and get in contact with water such as roof coverings, sealants, artificial turfs, wood-plastic-composites, or water pipes have been prepared according to the manufactures instructions and subjected to the Dynamic Surface Leaching Test (DSLT, liquid/area-relation 20 L m^{-2}) for monolithic and plane products or the One Stage Batch Test (OSBT liquid/solid relation 2 L kg^{-1}) for granular products. The eluates were chemically characterized and tested in the algae, daphnia, luminescent bacteria, zebra fish egg, and umuC test according to ISO standards. Biodegradability of eluates presenting a TOC > 10 mg L^{-1} was determined in the respirometry test (OECD 301 F). Most of eluates showed no or rather low effects in the aquatic ecotoxicity tests, but some were highly toxic (up to dilution factors of 1536). Biodegradability of most eluates was satisfying, but two eluates showed degradation extents below 50% in 28 d. None of the eluates was genotoxic in the umuC test. As a next step a guideline for the ecotoxicological assessment of construction products will be developed, if feasible, under CEN / TC 351. In autumn 2014 and early 2015 an interlaboratory round robin test is planned. Laboratories interested in participating are invited to get in touch with us. The results presented are part of the project FKZ 3712 95 309, funded by the German Federal Environment Agency.

TU289

Bio assays as integrated tool for the assessment of construction products

N. Bandow, Federal Institute for Materials Research and Testing / Contaminant Transfer and Environmental Technologies; S. Gartiser, I. Heisterkamp, Hydrotox GmbH; H. Mathies, BAM Federal Institute for Materials Research and Testing / Biodeterioration and Reference Organisms; U. Schoknecht, BAM Federal Institute for Materials Research and Testing

Construction products often contain complex mixtures of different chemicals such as stabilizers, softeners, flame retardants or residues from the production process. All these chemicals may be transferred to the environment during the whole life cycle of the products (construction, usage, demolition and recycling). Especially the contact with water can lead to leaching, and thus to contamination of the receiving water bodies and soils. For risk assessments usually leaching tests in the lab are performed and the concentrations in the eluates are compared with limit values subjected to the planned application. One major shortcoming of this approach is the limitation to a small set of selected chemicals, for which limit values for eluate evaluation have been derived in regulations. On the other hand it is impossible to identify and quantify all components in the eluates due to its complex nature. Bio assay may serve as an integrated tool for the determination of the environmental impact. This work exemplarily shows the results of granular ethylene-propylen-diene monomere rubber (EPDM), which is cross-linked with sulfur. This granular material is used for the construction of artificial turfs for outdoor usage. For determination of leaching properties 24 h batch tests with a liquid to solid ratio from 2:1 were performed. These eluates showed toxic effects as growth inhibition of green algae (*Pseudokirchneriella subcapitata*), acute toxicity to daphnia (*daphnia magna*) and inhibition of luminescence (*vibrio fisheri*). The chemical analysis of the regulated compounds and elements could not explain the observed effects. Only zinc showed elevated levels above limit values of the German groundwater protection directive. For screening of organic compounds the aqueous extracts were liquid/liquid extracted with solvent. The concentrated extracts were measured using GC-MS in the scan mode. Compounds were tentatively identified by comparing measured spectra with spectra from the library. Examples for identified compounds are mercaptobenzothiazole and its degradation products used for vulcanization, thiourea used as curing agent and piperidine derivatives used as stabilizers. This work was funded by Umweltbundesamt (UBA) under project no 371295309 and is a collaboration work of Hydrotox GmbH (S. Gartiser, coordinator), Hochschule für Technik Rapperswil, Institut für Umwelt- und Verfahrenstechnik (M. Burkhardt), BAM (U. Schoknecht) and ToxRat Solutions GmbH (M. Ratte).

TU290

Contributions of individual metals to equitoxic mixture toxicity

M. Kraak, University of Amsterdam; R. Korver, R. Vis, J. Schutt, K. Duinmeijer, UvA; S. Sjollema, University of Amsterdam

In contaminated ecosystems organisms are often exposed to a wide variety of toxicants. Yet, mixture toxicity is scarcely studied, especially the effects of complex mixtures. Moreover, complex mixtures may consist of compounds with different modes of action, likely not equally contributing to mixture toxicity. Therefore the aim of the present study was to determine the contributions of individual metals to equitoxic mixture toxicity. To this purpose the effects of Zn, Ni, Cu, Cd, Pb and Cu, binary metal mixtures and a mixture of these six metals on *Daphnia magna* were studied, with immobility after 48h of exposure as endpoint. Equitoxic mixtures were tested applying the TU concept. The EC_{50} values were 1890 µg/L for Zn, 1990 µg/L for Ni, 25 µg/L for Cu, 195 µg/L for Cd, 1030 µg/L for Pb and 8260 µg/L for Al. Some binary metal mixtures were concentration additive, but the Pb+Ni mixture was more than concentration additive. Hence, our results showed that the toxicity of metal mixtures cannot be predicted based on the toxicity of the single metals. The toxicity of the mixture of the six metals was additive (EC_{50} 1.2 TU (95% CI 0.9-1.5 TU), but we observed an inverse relationship between the slope of the dose response curve of the individual metals and the contribution of each metal to the mixture toxicity. It is concluded that differences in the slopes of the dose-response curves of the individual metals are an indication of their different modes of action and may be used to predict their contribution to mixture toxicity.

TU291

Field relevance of laboratory metal mixture toxicity tests

S. Sjollema, University of Amsterdam; R. Fernandez, K. Kloet, J. Kreuning, UvA; T. Peters, The University of Iowa / Department of Occupational and Environmental Health; A. Spelt, UvA; M. Kraak, University of Amsterdam

Water quality standards are generally based on single compound toxicity data, which contrasts with the ubiquitous environmental presence of mixtures of contaminants. Hence mixture toxicity testing is urgently required and indeed increasingly performed, but the field relevance of the outcomes is often not evaluated. Therefore, the aim of the present project was to study metal mixture toxicity and to evaluate if concentrations of individual metals contributing to mixture toxicity in laboratory tests are exceeded in contaminated ecosystems. To this purpose the effects of Zn, Cu, Pb, Al, binary mixtures, tertiary mixtures and the quarternary mixture on *Daphnia pulex* were studied, with immobility after 48h of exposure as endpoint. Equitoxic mixtures were tested applying the TU concept. The EC50 values were 2405 µg/L for Zn, 50 µg/L for Cu, 30000 µg/L for Pb and 14250 µg/L for Al. In the mixture toxicity experiments, more and less than concentration additive effects were observed, showing that the toxicity of metal mixtures cannot be predicted based on the toxicity of the single metals. The toxicity of the mixture of the four metals was more than concentration additive (EC_{50} 0.6 TU; 95% CI 0.5-0.8 TU) and we demonstrated that metals may contribute to mixture toxicity at concentrations that cause no effect when tested individually. The concentrations of Al and Cu present in the EC_{50} of the quarternary mixture were exceeded at a contaminated site in the Netherlands, providing evidence that metal concentrations that contribute to mixture toxicity in the laboratory actually occur at contaminated sites.

TU292

Uptake kinetics and toxicity of Cd, Cu and Pb mixtures in Asellus aquaticus using stable isotope techniques

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In the natural environment trace metals most often occur in different mixtures, in which metals can strongly interfere with each other, both at biological uptake sites and at the site of toxic action. The main objective of the current project is to study the competitive/inhibitory effects of Cd, Cu and Pb mixtures in the freshwater isopod *Asellus aquaticus* via water-only exposure, and relate these findings to observations in (sub)lethal metal toxicity. Stable isotopes (^{108}Cd, ^{65}Cu and ^{204}Pb) of the different elements were used to follow kinetics of metal uptake from solution in time (after 1, 2, 4 and 10 days). Exposure concentrations were 125 μg L^{-1} Cd (1.6 LC50), 1800 μg L^{-1} Cu (1.8 LC50) and 6400 μg L^{-1} Pb (0.8 LC50) respectively, which were added both separately (Cu, Cd, Pb), in binary (Cd+Cu, Cd+Pb and Cu+Pb) and in tertiary mixtures (Cd+Cu+Pb). Everyday mortality was checked and an *A. aquaticus* feeding assay was executed. Metal isotopes in tissue and water were determined using a High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS). Experimental data were fitted to either a linear or a hyperbolic function to estimate uptake rates. Our results indicate that ^{108}Cd uptake rates in *A. aquaticus* are significantly higher in the single Cd exposure treatment compared to all the mixture treatments, demonstrating the inhibitory effect of Cu and Pb additions (both singular and in mixture) on Cd uptake. No significant inhibition of both ^{204}Pb and ^{65}Cu uptake kinetics due to metal (Cd, Cu and/or Pb) addition was observed in the current study. No significant differences in feeding rate and mortality were observed between the mixture treatments after 10 days, although mortality in all treatments significantly increased compared to the control treatment. Highest mortality was observed in the single Cd treatment (46.7% mortality after 10 days exposure). A significant positive relation between Cd uptake rate and mortality was observed, however not for Pb and Cu. To conclude, the present study revealed a significant inhibition of Cd uptake rates in *A. aquaticus* after 10 days exposure due to the presence of Cu and Pb in the medium. Our results indicate that changes in Cd uptake rates can already result in decreased mortality after short term exposure via water despite the presence of other potentially toxic metals.

TU293

Environmental risk assessment of metal carboxylates

L. Claeys, K. Oorts, ARCHE

Metal carboxylate complexes are salts comprised of a metal atom and one or more organic acid (RCOOH) moieties. The metal ions can be monovalent or divalent and cover several groups from the periodic table: alkali metals (e.g. K), alkaline earth metals (e.g. Ca) and several groups of transition metals (e.g. Co, Zn, Mo, Mn and Zr). The carboxylic acids can vary in terms of chain length, differences in the degree of saturation and differences in branching. The fate and behavior of such salts in the different environmental compartments affects the way risk assessments are performed. Upon dissolution in aqueous media, a complete dissociation into free metal ions and carboxylic acid anion is expected at environmental and physiological relevant pH values. Both moieties then show their proper fate and behaviour in the environment, which may result in a different distribution over the environmental compartments (water, air, sediment and soil). The environmental risk assessment of metal carboxylate salts is therefore preferentially based on ecotoxicological data for both individual moieties. This approach further allows making maximal use of available data for the parent compounds. The selection of appropriate release factors and the use of the concentration addition method for combining results for the individual moieties significantly affect the outcome of the exposure and risk assessment. The Eurometaux metal specific Environmental Release Categories (spERC) can be used for the exposure assessment of the metal moieties. The release factors of the carboxylic acids can be based on the general Environmental Release Categories (ERC), however spERCs for specific sectors are also available, depending on the uses (e.g. CEPE for use as coatings).This poster will present an overall approach for risk assessment of metal carboxylates and discuss the implications of the selected strategy.

TU294

Exploring mixture toxicity of herbicides in rivers and its treatment by ecotoxicological models

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Pesticide contamination, in particular herbicidal loading, has been shown to exert pressure on Luxembourgish rivers. Such pollution can have direct impact on autotrophic species, as well as indirect impacts on freshwater communities through primary production degradation in macrophytes. This study aims to explore mixture toxicity of herbicides detected in rivers and its treatment by ecotoxicological models, using Luxembourgish rivers as case study. Current monitoring programs do not encompass the wide variety of pesticides available on the market, and existing impact evaluation assumes that the limited set of monitored pesticides represents the whole toxic pressure. Recent literature concerning pesticide mixture toxicity shows the validity of the 'concentration addition' concept, but lacks a link between laboratory experiments and field measurements. This study will employ a variety of measures in an attempt to breach these gaps. A combination of passive sampler field extracts (POCIS) and artificial spiked mixtures based on field extracts will explore the concentration addition concept through algal and macrophyte assays. These assays will use cell density, cell volume and photosynthesis inhibition as end-points to determine mode of action of individual contaminants and mixture constituents. Subsequent toxicity assays performed on rooted and floating macrophyte species will use the same pesticide profiles tested on algal species. The data derived from these experiments will be used to calibrate the ecotoxicological model AQUATOX, and to extrapolate these effects across time and different boundary conditions. Presented preliminary results include: (i) the spatial and temporal variability of pesticides extracted from POCIS samples; (ii) the implementation of algal toxicity assays using single contaminants and mixtures of pesticides.

TU295

Should tank mixtures be considered in evaluating the risk of plant protection products to aquatic organisms?

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The environmental risk assessment of plant protection products (PPP) is based on the products intended to be marketed. These products usually contain 1 to 3 active ingredients (AI) in formulation and are assessed for effects of the single substances as well as for the product as a mixture. If a risk is identified for aquatic organisms, distance requirements from edge-of-field to surface waters are usually based on the AI with the highest risk. In practice, different products are also applied simultaneously in tank mixtures to the crop. A risk assessment based on a single product might underestimate the effects of the products in a tank mixture. This could be the case if several the AI have a toxicity exposure ratio close to the trigger for a taxonomic group. Using Toxic Units and the concept of concentration addition, we investigated if risk assessments based on individual products are also protective for commonly used Swiss tank mixtures. In Switzerland different distances to surface waters to mitigate the risk to aquatic organisms are 6, 20, 50, 100 meters. Possible consequences of risk assessments of tank mixtures with regards to buffer strips are discussed in the poster.

TU296

Binary mixture toxicity of triclosan and carbendazim on Daphnia magna

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In the environment, mainly due to anthropogenic activities, compounds appear as complex mixtures and consequently organisms are exposed to different chemicals. Carbendazim and triclosan are examples of widely used compounds that end up on the aquatic system: carbendazim is a fungicide largely used in agriculture and triclosan has a biocide action and it's used in a variety of personal care products (e.g. toothpaste, shampoos, cosmetics, *etc*). When studying chemical mixtures, there are two main reference models that are used to predict toxicity: the Independent Action (IA), which assumes that chemicals have different modes of action (MoA) and Concentration Addition (CA) that assumes that chemicals have the same MoA. However, deviations from these reference models can occur, as synergism/antagonism, Dose Ratio (DR) or Dose Level (DL) dependencies. The aim of the present study was to investigate the effect of the binary mixture of triclosan and carbendazim on *Daphnia magna*. For that, currently used ecotoxicity tests were carried out (immobilisation, feeding inhibition and reproduction tests) and the comet assay was also used to infer if DNA damage was the main mode of action playing a role in toxicity. The MIXTOX model was used to evaluate mixture effects. It was observed that some endpoints measured on *D. magna* followed the reference models (CA and IA) and others presented deviations from the conceptual models (synergism, antagonism or DR and DL dependency). A DL deviation showed to be the best description to the immobilisation data, with synergism at low doses and antagonism at high doses of the chemicals; in the feeding inhibition test, the 24 hours exposure data presented an antagonistic pattern; finally, a synergistic pattern was observed for the reproduction data. Comet assay revealed that, when daphnid cells were exposed to both compounds an increase in DNA damage is observed; in the mixture toxicity the DR deviation was the best fitted deviation, with synergism, except when the toxicity of the mixture is mainly caused by carbendazim (here there is antagonism). This pattern was not expected as in single toxicity tests carbendazim induced aborted eggs, possibly due to effects on the mitosis during embryogenesis. In conclusion, and as both compounds appear in the environment together, this study will provide additional and useful information to predict risk assessment.

TU297

Evaluation of Genotoxic Effects of Ayahuasca in Wistar Rats

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Ayahuasca (or hoasca) is a psychoactive beverage produced by the concoction of two plants (*Bansteriopis caapi* and *Psychotria viridiis*). It is traditionally used by various Amazonian indigenous populations in shamanic rituals and since the 30`s it has been introduced as part of religious rituals in non-indigenous population of Brazil. The ingestion of this infusion causes hallucinogenic effects and psychomotor reactions due to it agonist effect in serotonin receptors. The increased consumption of Ayahuasca by non-religious groups, in great urban centers represents a potential public health problem. In addition, very little is known about the possible genotoxic effects of this concoction in humans. Ayahuasca infusion kindly provided by União do Vegetal (UDV)-Brasília, was lyophilized and stored. The lyophilized product was weighted, diluted in filtered water and administered to animals by gavage (50 Wistar rats - 25 males and 25 females). 30 hours after

treatment, the animals were euthanized by CO_2 inhalation. Bone marrow and blood were collected for analysis. Comet assay (CA) and DNA fragmentation by flow cytometry (FC) were used to detect possible DNA damage induced by acute exposure of Ayahuasca. Experimental design included 5 groups: positive control with doxorubicin (CG+), negative control with filtered water (CG-) and 3 different doses of Ayahuasca (1X, 15X and 30X the ritual dose). In CA, the total damage index did not show a genotoxic potential of Ayahuasca in the ritual dose (1X = 150ml / 70Kg). Although we found DNA fragmentation even at the lowest doses, there was no significant difference between treated groups and negative control. These results suggest that the ritualistic use of Ayahuasca is safe in relation to DNA damage. Acknowledgement: FAPDF, CAPES and UDV.

TU298

Sensitivity of hypogean and epigean freshwater copepods (Crustacea Copepoda) to agricultural pollutants: single toxicants and mixtures

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Widespread pollution from agriculture is one of the major causes of poor freshwater quality currently observed acrossEuropeand worldwide. Nutrient loads (nitrogen and phosphorous) from fertilizers and pesticides are known to adversely impact freshwater ecosystems, both surface- and ground water. The Crustacea Copepoda are by far the most abundant and species-rich taxon in ground water and they are consistently represented in ecotonal environments also, as in the hyporheic zone. The direct impact of agricultural pollutants on freshwater biota has been addressed in several studies by means of laboratory bioassays. However, the ecotoxicological research concerning freshwater copepods is scarce for epigean species and almost non-existent for the hypogean ones. Moreover, when available, ecotoxicological studies have been performed considering the effect of toxicants taken individually. Actually, this approach does not reflect the conditions in the field high concentrations of both N-fertilizers and pesticides co-occur. In this study we assessed the acute (at 96h) sensitivity of adults of a hypogean and an epigean species, both belonging to the Crustacea, Copepoda, Cyclopoida, Cyclopidae, to two agricultural toxicants: the ammonium nitrate and the herbicides Imazamox. Both chemicals are widely used for cereal agriculture inEurope. We tested the sensitivity considering the LC50 with mortality endpoints for individual and combined (a mixture solution of ammonium nitrate and Imazamox) toxicant concentrations. The hypogean species was more sensitive than the epigean one to both chemicals and their mixture. Ionized ammonia proved to be more toxic than the herbicide Imazamox for both species. However, the LC50 of both chemicals were lower than the actual standard law limits for good freshwater quality status defined by the Water Framework Directive (2000/60/CE). The effect of the mixture, of the two toxicants was fairly synergic. Concerning ionized ammonia, the LC50-96h in the mixture was higher than the law limits for both species. According to these results, the actual law limits for the good quality of freshwater bodies should be revised accodingly by authorities in charge of water management.

TU299

Impact of pollutant mixtures on feral fish – Biomarker analysis within the SOLUTIONS EU FP7 project and Joint Danube Survey 3

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There is increasing awareness that toxicants may significantly contribute to the deterioration of the ecological status in European water bodies and may endanger the sustainable use of this crucial resource. Nevertheless, required regulatory measures by the competent authorities are more difficult, or often impossible. Biomarkers may play an important role in the detection and diagnosis of the impact of toxicants on biota. Thus, within the 7th EU RTD Framework Programme project SOLUTIONS (Solutions for present and emerging pollutants in land and water resource management) we are developing a multi-biomarker approach in feral, caged and laboratory fish in order to link effects on different levels of biological organization to chemical contamination and to identify the impacts of pollutant mixtures. This approach has been demonstrated in the frame of Joint Danube Survey 3 (JDS3). During the JDS3, which took place from the August 12th to 26th September 2013, fish samples including two different fish species (up to 10 individuals per site and specie) with different habitat preferences have been collected at 32 sampling sites along the Danube River from Regensburg (Germany) to Tulcea (Romania) and were immediately dissected and prepared for further biomarker analysis in the laboratory. The biomarker battery includes a wide range of biological endpoints such as mutagenicity, enzymatic activity of the detoxification metabolism, chemical analysis of xenobiotic metabolites in bile liquid and accumulated pollutants in muscle tissue as well as pathological alterations in liver tissue. The biomarker results are discussed in the context of extensive chemical screening that has been performed in corresponding water samples after *in situ* large volume solid phase extraction.

TU300

Cytotoxic and genotoxic effects of water and air samples from polluted areas in Kosovo

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Environmental pollution in Kosova is one of the major issues in the current phase of the development. Reports on the state of the environment warned for an "unacceptable sources of air pollution'' whereas river and groundwater quality is affected by pollution from untreated waste water from the industry. The main "contributors" of this pollution are located in Obiliq (coal Power Plants), Mitrovica (smeltery of lead and zinc), Drenas (smeltery of iron and Nickel) and Hani i Elezit (cement factory). Prishtina (the capital city of Kosova) is also heavily influenced by the pollution emitted from Power Plants in Obiliq. In this study which is supported by an EU funded project ("Environmental pollution in Kosova: potential genotoxic effects and related human health risks"), we are investigating the cytotoxic and genotoxic potential of air and water samples taken from these localities in plant (Allium test) and animal cells (rat hepatocytes). The preliminary data show cyto- and genotoxic effects of air and water samples from polluted localities in rat hepatocytes (as regard to the frequency of necrosis, apoptosis and micronucleated cells). The frequency of chromosomal aberrations in Allium test was shown to be elevated too. We expect that the obtained data and further reliable assessment of the potential long term risks is expected to have an impact on determination of risk factors and raising the awareness on the need for preventive and remediation measures.

TU301

Toxicological effects of mixtures of chemical pollutants at EQS concentrations

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Mixtures of 14 or 19 chemical substances of concern for the contamination of surface waters, each present at concentrations equivalent to their environmental quality standard (EQS) have been produced as a reference material and tested using biological effect-based tools. Around 30 bioassays covering the most relevant ecotoxicological endpoints and 13 organisms from different trophic levels have been included in the exercise in which 16 laboratories have participated. The chemicals selected for the mixtures were the substances atrazine, diuron, isoproturon and simazine (herbicides), benzo[a]pyrene and fluoranthene (polycyclic aromatic hydrocarbons), cadmium and nickel (metals), DEHP (plasticizer), 17β-estradiol (naturally occurring estrogen), 4-Nonylphenol (surfactant), diclofenac (pain killer), chlorphenvinphos and chlorpyrifos (insecticides), and the emerging compounds bisphenol A, carbamazepine, sulfamethoxazole, triclosan and DEET. The results from this exercise showed a general comparability between different bioassays targeting the same biological endpoint and classes of substances. The mixtures of chemicals at EQS concentrations originated effects on a few of the methods. These include changes in the algal-bacteria composition in a marine microcosm, effects on immobilization in Daphnia magna, fish embryo toxicity and effects on frog embryo development. Additionally, increased expression on reporter genes linked with oxidative stress was observed in both *C. elegans* and *E. coli*. An addictive effect was apparent for methods sensitive to estrogenic compounds and photosystem II inhibitors. The results indicate that some precaution on the chemical mixture assessment should be taken even in cases where individual compounds are present at seemingly harmless concentrations.

TU302

Mixtures of bioactive compounds associated with cyanobacterial water blooms

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Organisms in the environment are exposed to complex mixtures of both natural and anthropogenic compounds. Specific types of these mixtures might be associated with occurrence of cyanobacterial water blooms in freshwater environment. The compounds associated namely with water blooms of greater densities can include known cyanotoxins, but also other bioactive cyanobacterial metabolites as well as man-made contaminants present in surface water and/or accumulated in water bloom's biomass. Some of them might possess biological activity, which can lead to many negative effects in exposed aquatic organisms, e.g. endocrine disruption, developmental and reproductive disorders. Little is known about some of these compounds, their interactions and mixture effects. Presented work evaluates endocrine disrupting potential of extracts of water bloom biomasses and surrounding surface water sampled in several ponds and dams during summer 2012. One location was sampled periodically every two weeks to investigate the seasonal variability of the bioactive compounds' occurrence. Water samples were concentrated by solid phase extraction (SPE), cyanobacterial biomass was extracted by ultrasonic extraction. Endocrine disrupting activity was investigated by *in vitro* bioassays using mammalian cell lines with appropriate receptor and trans-activated reporter gene for estrogenic, retinoid and dioxin-like activity. Results of bioassays show that there are compounds with estrogenic, dioxin-like and retinoid activity, both in water bloom biomass and in the surrounding water. Also, the extractability of these compounds from biomass varies greatly when different methods/solvents are used. The activities differ among sites and vary also in time on the same site. Some of the known pollutants that could contribute to the observed biological potencies were analyzed to determine their contribution. The risks associated with the occurrence of the mixtures with studied types of bioactive compounds in the freshwaters with water bloom development will be discussed. The work was supported by the Czech Science Foundation grant No. GACR P503/12/0553.

TU303

Critical Evaluation of USEPA's Toxicological Assessment of Benzo(a)pyrene & PAH Mixtures

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USEPA's draft toxicological assessment of Benzo(a)pyrene (BaP) was released for public review with comments accepted until November 2013. The document proposed a new Oral Slope Factor of 1.0 $(mg/kg\text{-}day)^{-1}$. USEPA also presented four new values: Inhalation Unit Risk (0.0005 $(\mu g/m^3)^{-1}$), oral Reference Dose (0.0003 mg/kg-day), inhalation Reference Concentration (0.000002 mg/m^3), and a first ever Dermal Slope Factor (DSF) (0.005 $(\mu g/day)^{-1}$). These toxicity factors for BaP have far reaching implications because BaP is the *indicator* PAH and is used to assess the risks posed by all PAH mixtures using USEPA's Relative Potency Factors (RPFs) for other potentially carcinogenic PAHs. The converted DSF is 350 $(mg/kg\text{-}day)^{-1}$ for a 70 kg adult, which makes ingestion exposure moot. This talk provides a critical review of the derivation of the 5 toxicity factors. The DSF is scientifically inappropriate because it presumes that mouse skin is predictive of human skin, but ignores the large epidemiological literature on coal tar pharmaceutical users, which shows that highly exposed humans do not contract skin cancer. The DSF is also based old, poor studies in which BaP dosimetry was not carefully quantified. More importantly, several studies were ignored without cause and of the dozen studies that were considered, the two studies that were documented to have exceeded the Maximally Tolerated Dose were chosen as the basis of the DSF. The dose-response modeling was also flawed in that it (a) averaged doses over a longer period that the animals' lifespans, (b) ignored the documented *depot* effect for repeated skin doses of BaP, (c) used selected dose-response models, (d) ignored USEPA's own guidance on assessing model fit, and (e) ignored the clear threshold in the mouse skin dose-response data. The other toxicity values are similarly critiqued. Finally, the implications are discussed. For instance, if the proposals are adopted, the ingestion pathway for cancer risk assessment will be insignificant and dermal risks will dominate. The Regional Screening Level will drop to 3 parts-per-billion, and it will be predicted that 10% of all skin cancer in the US will have been caused by exposure to PAHs in soil, despite the fact that dermatologists have concluded that most skin cancer is caused by UV exposure. The proposed toxicity factors for BaP and the proposed RPFs for 25 PAHs will be validated by comparing estimated and actual tumor incidence rates from studies of PAH mixtures.

TU304

TIME-DEPENDENT MIXTURE TOXICITY ASSESSMENT FOR AQUATIC ORGANISMS

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For aquatic organisms, the risk from combined exposure to more than one active substance can be addressed by product data which are required for acute effect data on fish, aquatic invertebrates as well as for algae and aquatic plants. However, acute effect data for the formulation are only relevant for drift entry, whereas other entry routes cannot be addressed by data based on the ratio of active substances in the product. For run-off and drainage entry, predictions are to be based on active substance data. Concentration addition in the regulatory context of ecotoxicological risk assessments is widely accepted as a reasonably conservative model to assess the potential for combined effects of more than one active substance. A practicable approach is the evaluation of combined effects based on Toxicity/Exposure Ratios for all actives. The cumulative risk, however, is conservatively based on the assumption of peak exposures occurring simultaneously, whereas the predicted exposure levels for active substances might differ in time of maximum occurrence depending on their environmental fate characteristics. In such cases a detailed analysis of the time-course of exposure can be used as refinement option. An example for a time-dependent additive mixture toxicity assessment is provided for the entire simulation period as obtained from FOCUS higher tier modelling. The analysis is conducted using a new software tool presented by Weber in the poster presentation "Mixture-toxicity exposure assessment in FOCUS surface water scenarios".

TU305

Preliminary studies for application of the WHO/IPCS framework on multiple chemicals to regulatory environmental risk assessment

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From the standpoint of environmental risk assessment for chemicals management through environmental regulation, component-based approach should be considered for combined exposure to multiple chemicals. Preliminary studies are being conducted aiming at prioritizing candidate groups of chemicals to be subjected to assessment of combined exposure referring to the WHO/IPCS framework published in 2011. The goals of the studies are to develop a framework and to compile a guidance document on environmental risk assessment of combined exposure to multiple chemicals for internal reference within the ministry responsible of environmental protection. In the first study, ninety-six substances were selected from the existing list of candidate chemicals for setting "Environmental Quality Standards for Water for conservation of aquatic lives". They were categorized considering the similarity of their structure referring to the information on Category Chemicals in the OECD Existing Chemicals Database, and phthalates and alkylphenols were selected. Tiers 0 and 1 of the WHO/IPCS framework were partly modified for obtainable data on both environmental concentration and ecotoxicity and screening-level environmental risk assessment was attempted with the framework. For both groups of chemicals the result "Yes, no further action required" was not obtained and "No, continue with iterative refinement as needed" was concluded. The second study is attempted to chemicals detected in the aquatic environment in Japan. Results of the ministry's "Environmental Survey and Monitoring of Chemicals" over the past ten years are

listed and organized considering simultaneous exposure to aquatic organisms. Applicability of the lower tiers of the WHO/IPCS framework to screening-level environmental risk assessment of the selected chemicals is to be examined. This abstract summarizes the situation as of the end of November 2013. Updated status of the preliminary studies is to be presented and discussed.

TU306

REACH-compliant risk assessment of a naturally-sourced complex substance

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Under regulations such as REACH the term 'substance' has a specific meaning such that a single name (and associated CAS/EC number) can be used for a product containing a large number of individual chemical compounds, termed constituents. The term 'UVCB' (unknown or variable composition, complex reaction product, or biological material) is used in REACH. In risk assessment a number of considerations have to be made to decide the strategy, but the basic premise is that each constituent will, following any release to the environment, behave independently to the others. However, many questions need to be addressed, which are exemplified in this poster. Concerning properties, questions for consideration include: Can constituents be grouped into blocks, making possible the use of the 'hydrocarbon block' method? What are the principles of blocking? Are any measurements on the whole substance of any value? How much information about the individual constituents is available in terms of basic properties and mode of action? Can co-operative effects between constituents be envisaged? How can the overall characterisation of risk be achieved, for each protection goal? Can QSAR be used to improve the assessment? How can uncertainty be addressed? For substances which have a natural origin, the constituents are inevitably already present in the environment. This creates another layer of challenge to the risk assessor, because the approach to assessment of a predicted no-effect concentration becomes more complex. In respect of release to the environment, it is also necessary to examine the possibility that some constituents will be released, proportionately, more than others. This is also discussed in terms of solubility and volatility properties. The consequence often is that each constituent or block can be assessed, but the summation of risk characterisation ratios should not be performed without extensive justification. When the range of protection goals is pelagic, benthic and terrestrial compartments, numerous factors affect these exposures differently. For protection of the food chain, including humans, the application of any available data on effects in mammals is also challenging. Substances such as petroleum hydrocarbons, plant-derived oils and resins are discussed. These illustrate the problems that can arise when blocks have very different properties.

TU307

Use of the "Maximum Cumulative Ratio" as a tool for prioritization of mixtures exposures to Plant Protection Products (PPPs): a case study based on surface water monitoring in the USA.

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Environmental exposure is typically characterised by exposures to several plant protection products (PPP) from multiple sources, such as urban and agriculture use. While current regulatory programs evaluate risks on a chemical-by-chemical basis there is considerable interest in assessing the impact of cumulative exposures. Hence, the risk assessor would benefit from knowing when mixtures itself is a concern and when the assessment can just focus on the safety of the individual chemicals that make up the mixture. The Maximum Cumulative Ratio (MCR) is a tool for investigating the magnitude of the toxicity received by a receptor that is missed if a cumulative risk assessment is not performed (Price and Han, 2011). The method evaluates the contribution of each substance to the overall effect of the cumulative exposures to an environmental receptor with the goal of identifying if the toxicity is driven by one substance or if multiple components make significant contributions to the combined toxicity of the individual's exposure. The following case study evaluates the risk of pesticides measured in approximately 3000 surface water samples across the USA between 1992 and 2001 (USGS,2006; Price and Han, 2011). The risk from exposure to individual PPP was calculated based on the reported concentration and the published aquatic life benchmark (EPA website). The risk of the mixture was conservatively derived by assuming additivity of effects and the MCR were subsequently calculated to discuss the relevant of mixture risk assessment. Considering that mixtures assessed comprise substances with known specific mode of action and defined target species, the analysis was performed separately for three trophic levels. Such an analysis would be considered a Tier 2 assessment under the WHO framework for assessing combined exposures (WHO, Price et al., 2011). While the relevance of pesticide exposure is often discussed in relation to the number of detection in environmental samples, the MCR supports a risk based approach in reducing exposure to the aquatic environment by identifying samples where single substances are the drivers of mixtures' toxicities. This approach also allows the assessor to identify those mixtures where toxicity cannot be determined by evaluating on a chemical-by-chemical basis and will need additional review.

TU308

Research recommendations to improve an environmental integrity and human health risk analysis in a groundwater-supplied borrow-pit lake ecosystem

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Borrow-pit lake ecosystems are formed after road construction activities and are designed to support recreational beneficial uses. Resource agency concerns about the lack of fish reproduction and potential human health issues associated with the consumption of fish from a groundwater-supplied borrow-pit lake ecosystem were evaluated. Water and whole-body fish samples were collected and analyzed using standard methods for selected ion, metal, and pesticide concentrations. In a preliminary risk assessment the concentrations were compared to existing United States water quality criteria (WQC, to protect aquatic life) and human consumption thresholds (USEPA IRIS database). Concentrations of concern in the water included total Se at 5.75 ppb (WQC 5 ppb), and chloride at 376 ppm (WQC 230 ppm). Elements of concern, based on human health issues associated with the consumption of fish included Zn, As, Se, and Hg. Average whole fish tissue concentrations (std. dev.) for Zn, As, Se, and Hg were 128 (38), 0.32 (0.30), 0.84 (0.12), and 0.26 (0.12) mg/kg wet weight, respectively. The IRIS human consumption thresholds for Zn, As, Se, and Hg are 21, 0.021, 0.35, and 0.007 mg/day for a 70 kg adult, respectively. All organics were below detection limits. The results and any conclusions were confounded by the lack of quality assurance and quality control data, and lack of appropriate replication and experimental design. The elevated water and tissue Se concentrations could explain the lack of fish reproduction in this system. The preliminary data suggest potential human health issues associated with the consumption of fish. However, the noted deficiencies in the data collection and the lack of an exposure assessment prevented calculating the human health risk and therefore recommending appropriate risk management methods. A more through risk analysis is recommended including improved sampling design, data quality objectives with appropriate quality assurance and quality control protocols, analysis of fish fillets, and an exposure assessment to validate the preliminary conclusions.

TU309

Devils in the tails - Assessing mixture toxic pressure (msPAF) and chemical footprinting for emerging chemicals

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The EU FP7-project SOLUTIONS aims to provide management perspectives (solutions) for present and future emerging pollutants in land and water resources management. This implies focus on the future emissions scenarios of known and unknown compounds to aquatic systems, early focus on abatement options, and an approach in which alternative abatement strategies can be evaluated at the landscape-level of complex mixture impacts. In relation to that, we developed a methodology for deriving a chemical footprint, to quantify, at least in a realtive sense, the expected mixture impacts which result from landscape-level emissions of chemicals. Given the fact that future emission scenarios do not allow 'measurement of impact', model tools are applied in SOLUTIONS to explore future mixture impacts. Our explorative assessment of the expected impacts of mixtures of known and as yet unknown chemicals makes use of the concept of mixture toxic pressure, as derived using ecotoxicity data for various species, the Species Sensitivity Distribution (SSD) model, and mixture impact modeling. Our explorations showed that there are devils in the tails, that is: the mixture impacts resulting from low-level exposures rely heavily on the tails of the SSDs, where the uncertainties in the expected ecotoxicity are high. Explorations demonstrated that chemical footprinting for mixtures of emitted chemicals requires specific focus on ecotoxicity and uncertainty in case of low-level exposures. We propose a novel tail-of-SSD-related solution, which can be applied when alternative chemical management scenarios are to be compared and summarized via chemical footprinting methods. The method is based on using prior information on the known ecotoxicities of compound and compound groups to limit ecotoxicological potencies in SSD tails.

Chemical pollution in sustainable management of aquatic ecosystems – challenges and approaches from a Swiss perspective (P)

TU310

Biocides in combined sewer systems: dry and wet weather occurrence and sources

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In recent years more and more houses are equipped with biocide-containing façade coatings or fungicide treated wood. Typical examples are triazines as terbutryn; carbamates as carbendazim; and phenylureas as diuron for paints and renders and triazoles as propiconazole as fungicides for wood preservation. These biocides are leached out of the material while being hit by wind-driven rain and, hence, are detectable in combined sewer systems during wet periods with concentrations up to several hundred ng L^{-1}. However, the present study of both dry and wet weather influent sampling of five wastewater treatment plants in Denmark and Sweden showed that these compounds also occur during dry weather periods, when leaching from façade coatings can be excluded as source. The concentrations were on the same order of magnitude as during wet weather also reaching up to several hundred ng L^{-1}. For propiconazole noteworthy high concentrations (up to 4.5 μg L^{-1}) were detected at one of the wastewater treatment plants. Based on time resolved (12 x 2 h) sampling some presumptions about possible sources for the biocides were made. During wet weather the highest mass load per 2h-interval of up to 250 mg $2h^{-1}$ (propiconazole) was detected when the rain was heaviest, supporting the hypothesis that the biocides are washed off by wind-driven rain. In contrast, the mass loads of a dry weather day followed the human activities, meaning they were highest in the morning and evening hours (up to 100 mg $2h^{-1}$), as well as substantially lower during the night. Only the propiconazole emission showed a different behaviour: the mass load peaked in the late afternoon with a slow decline during the following eight hours, resulting in a total mass load of 17 g d^{-1}. This raises the assumption that the emission is caused by one single point source. Since propiconazole is also used as plant protection product this application may originated in inappropriate cleaning of spray equipment for agriculture or gardening. Overall, this study showed that about 20– 40 % of the total biocide emissions were emitted during dry weather, for propiconazole even 92 %.

Environmental OMICs: high-throughput strategies to decipher mechanism of response to stressors (P)

WE001

Tracking multilevel effects of nickel in Porcellionides pruinosus (Isopoda): from genomics to organisms

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Isopods represent keystone detritivorous, widely exploited for ecotoxicological assessment. Although they represent key places within the food-chain, there is almost no information regarding their genetic code, their metabolic pathways or key metabolites, providing adaptations to handle high contents of metals. In fact their unique processes of accumulation and excretion that occur in hepatopancreas cells and enable the assimilation of high levels of metals from the environment are still very poorly understood. The terrestrial isopod *Porcellionides pruinosus*has shown highly tolerance to metal contaminated environments that may reflect adaptions at various levels of biological organization. Moreover, there is a lack of functional genetic information for isopods. It was for these reasons among others that this species was chosen as a candidate for our current analyses. Furthermore, there is little information on mechanistic toxicology underlying the isopods response to nickel exposure. The global aim of this work was to evaluate and assess the effects of a metal (nickel) within different organization levels, starting at the detoxification pathways (enzymatic biomarkers) and energy related costs associated, along with the physiological alterations at the individual level (mortality). From this starting point, a metabolomics analysis (using ^{1}H-NMR) was performed along with a gene expression analysis (qRT-PCR and transcriptome analysis). The toxicity of the metal nickel to terrestrial isopods was evaluated within a multi-organizational level and the key pathways and mechanisms involved in the metal toxicity were determined. A "line" that connects all the evaluated levels could be found and some adverse outcome pathways (AOP) could be defined from the results. Within the mechanisms involved, oxidative stress pathways were determined to be one of the central mechanisms responsible for dealing with this stressor. But not only expected pathways like this one were determined to be involved; in fact several metabolites and genes related to moulting, cell division and DNA damage handling seem to be also affected. With the following development of this study it was possible to better understand how this stressor affects the organisms and how they dealt with it and return to a "hormetic status". A global view of the detoxification processes that they undergoe is presented for further studies, and serving also as reference for other studies.

WE002

Toxicogenomic mechanisms of 2,2-bis(bromomethyl)-1,3-propanediol using E.coli knockout mutant screen

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2,2-bis(bromomethyl)-1,3-propanediol (BMP) is a brominated flame retardant widely used in polyurethane foams and unsaturated polyester resins. The widespread production and slowly degraded of BMP raise public concern about the potential risk of BMP to human and wildlife. Previous studies have shown that BMP induced DNA damage associated with oxidative stress. However, little is known about the toxicogenomic mechanisms of BMP. In this study, molecular mechanisms of BMP were assessed by high-throughput *Escherichia coli (E.coli)* genome-wide knockout library which contains 3898 clones of single gene-mutants, nearly 90% of E.coli genome. Each clone lacks a single gene. BMP inhibited wide-type E.coli growth with a 24h median effect concentration (EC50) 1615.47±35.59mg/L. Through the whole library screening with the concentration of wide-type EC50, 65 hits were identified, including 34 more sensitive clones and 31 more resistive clones using the median absolute deviation selection as cutoff (z=3). Then the selected hits were further verified by cytotoxicity test with full concentration range. The transcriptional network of these hit genes was developed to identify the key regulatory modules modulated by BMP in the network using Cytoscape software. The gene ontology (GO) analysis suggested that the hit genes were focused on metal ion-binding, DNA-binding, ATP-binding and some protein biosynthetic process pathway. The underlying mechanism of the cytotoxicity induced by BMP was also assessed by the metabolic pathways affected. **Keywords**: BMP, E.coli, knockout, high-throughput

WE003

Proteomic analysis in Daphnia magna exposed to Lead (II) acetate trihydrate and Atrazine for potential biomarkers screening

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ABSTRACT In these decades, heavy metals and pesticides are main factors causing water pollution via industrial waste. However, it still exists a limitation of well understanding about how these toxics effect the aquatic organisms and are there any changes in gene/protein expression due to environmental stress or not. In this study, acute toxicity tests were performed according to EPA protocol (2002) to assess the impacts of Lead (II) acetate trihydrate and Atrazine on aquatic species using a typical freshwater flea *Daphnia magna*. Besides, the proteomic profile of treated *D.magna* (LC20) was examined with a pH range from 3 to 10 using 2-dimensional gel electrophoresis (2DE) method and then analyzed with Progenesis software to explore the differentially expressed proteins (DEPs) compared with control organisms. The results showed that there were some up- and down-regulated proteins in *D.magna* responsed to these toxic chemicals. The changed spots due to each chemical can be used as novel biomarker candidates to detect these heavy metal and pesticide. ***Keywords***: *Daphnia magna*, Lead (II) acetate trihydrate, Atrazine, acute toxicity test, proteomic.

WE004

Proteomic response of Unio pictorum mussel to a mixture of glyphosate and microcystin-LR

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Freshwaters are regularly subjected to several contaminants including cyanobacterial toxins and pesticides. Among these contaminants, microcystin-LR is one of the most toxic and common cyanobacterial toxins found in freshwaters whereas glyphosate is the active ingredient of a widely use herbicide. As filter feeders, freshwater mussels are particularly exposed aquatic animals. Like many native bivalve species, *Unio pictorum* suffers from a continuous decline in Europe. Previous studies indicated that it could be more sensitive to microcystin than the zebra mussel, an invasive species. In order to get a deeper insight of its response to contaminants, *U. pictorum* was exposed to either 10 μg/L of microcystin-LR or 10 μg/L of glyphosate or a mixture of both. Proteins of the digestive glands were extracted and analyzed by DIGE. Gel analysis revealed 103 spots with statistical variations and the response seems to be less reactive towards glyphosate than to microcystin-LR. Specific spots have variations only when exposed to the mixture showing that there is an interaction of both contaminants in the responses triggered. The proteins of thirty spots have been identified. They belong mostly to the cytoskeleton family but proteins of the oxidative pathway, detoxification and energetic metabolism were affected either by glyphosate or microcystin-LR or by the mixture. These results demonstrate the importance to study contaminants at low concentrations representative of those found in the field and that multicontaminations can lead to different responses pathways.

WE005

Effects of the antifouling biocide tralopyril on the proteome of the mussel Mytilus galloprovincialis

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The growth of unwanted organisms on submerged surfaces leads to faster degradation of underwater equipment, increasing the maintenance costs. Antifouling paints are commonly used to prevent it. Although this approach is economically relevant, a special attention should be given to the risk assessment of booster biocides used to increase paint efficacy. Under the new Biocidal Products Regulation (BPR, Regulation EU No. 528/2012) the authorization of these compounds is becoming more restricted. Therefore, the need for environmentally friendly alternatives is rising. Tralopyril is one example of such novel biocides that has been marketed as a safer alternative and is already in use. However, the knowledge on its toxicity towards aquatic organisms and its mode of action is still insufficient. We performed a study aiming to i) characterize the acute and chronic effects of tralopyril on the proteome of the mussel *Mytilus galloprovincialis* by using global proteomics analysis and ii) understand if a recovery occurs after depuration. For this, mussels were exposed to tralopyril (1μg/L) during 30 days and then transferred to clean water where they were kept for 10 days. Three pairs of gills were collected per condition (control, solvent control and tralopyril) at different time points (after 2 (T2), 5 (T5) and 30 (T30) days of exposure, as well as after the depuration period (T40)). For protein extraction, three individual gills per condition and time point were pooled together. Proteins were digested with trypsin and the resulting peptide mixture was analysed using mass spectrometry-based Multidimensional Protein Identification Technology (MudPIT). Differentially expressed proteins were identified using a label-free approach based on spectral counting and G-test. Preliminary results point towards oxidative stress caused by tralopyril exposure. This study will contribute to the understanding of molecular mechanisms of tralopyril toxicity and may result in the identification of new biomarkers of exposure. Keywords: tralopyril; shotgun proteomics, *Mytilus galloprovincialis*

WE006

Protein expression of Pacific Oysters Crassostrea gigas exposed in situ to effluents containing sanitary sewage, Florianópolis, SC

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The composition and concentration of substances in effluents are quite complex and difficult to measure, being characterized by a mixture of domestic sewage, hospital waste and run-offs from gas stations and small industries. Among the bivalve mollusks most commonly used as sentinel organisms in monitoring programs, oysters *Crassostrea gigas* hold a prominent position, as they are the most cultivated and consumed marine mollusks worldwide. Proteomic analysis can be used as tools in environmental toxicology, by reflecting alterations in protein expression through environmental adaptations and providing a useful framework for the development of new biomonitoring tools complementary to genomic studies. Proteomics will thus help reveal particular alterations at the proteome level that can be further associated to the different types of contaminants present in sanitary sewage. So, the aim of this study was to determine changes in protein expression in the digestive gland of oysters *C. gigas* transplanted from a control farming area (SAM) to two areas of mollusks farming (LIS, RIB) and to one polluted area with sanitary sewage (BUC) for a period of 14 days. Proteins expression profiles of oysters from the polluted site were compared to those from the two cultivation sites (BUCxLIS; BUCxRIB) using two-dimensional electrophoresis to identify differentially expressed proteins. Proteins more drastically altered were excised and analyzed by mass spectrometry, where five were putatively identified. The identified proteins in this study are related to the cytoskeleton (CKAP5, ACT2), ubiquitination pathway conjugation (UBE3C), G protein-coupled receptor and signal transduction (SVEP1) and cell cycle/division (CCNB3). Among these proteins, CKAP5 showed higher expression in oysters exposed to BUC in comparison to the two areas of cultivation, while proteins ACT2, UBE3C, SVEP1, CCNB3 had a lower expression in the same organisms. A possible relationship was observed between the proteins expressed in higher quantity CKAP5 and proteins in lesser amounts ACT2, and UBE3C CCNB3. These results suggest that these changes might lead to DNA damage, apoptosis, and interference with the immune system in oysters *C. gigas* exposed to sewage. Nevertheless, the changes in ACT2, CKAP5, UBE3C, CCNB3 and SVEP1 should be better studied and considered in future studies with oysters exposed to urban sewage.

WE007

Metaproteomic analysis of Baltic soil exposed to human pharmaceuticals: next generation environmental assessment

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Henric Froberg[1], Jacob Kuruvilla[1], Gabriela Danielsson[2] and Susana Cristobal[1,3]

Environmental assessment has traditionally focussed on evaluating the environmental quality by measuring abiotic components. We hypothesise that estimation of the relative abundance of protein families from a complex community sample could be utilised as an environmental assessment tool that can detect changes in biodiversity. The proteins that vary in abundance from common to rare are the candidates with functional relevance in the assessment. We have tested this hypothesis by performing a metaproteomic analysis from the bacterial community living in Baltic soil. The sediment were selected for

WE008

Assessment of the toxicogenomic potential of complex industrial wastewater effluents from a treatment plant in Germany

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Rivers are of major importance for the human society, and strong contributors to biodiversity. Nevertheless, worldwide most rivers are heavily contaminated by pollutants of anthropogenic origin. Effluents from wastewater treatment plants represent a major source for discharge of such xenobiotics into the environment. For the risk assessment of complex environmental mixtures, causal links between chemical contamination and observed biological effects are indispensable. Effect directed analysis combines fractionation and chemical analysis with toxicity testing in bioassays, which finally leads to the identification of those compounds responsible for the toxic potential of the complex sample. In this process toxicogenomics can help understanding modes of action of detected toxicity and give information about which components present in the environmental sample are contributing to the toxic effect. The municipal treatment plant Bitterfeld-Wolfen treats wastewater of 18 communities and the Bitterfeld-Wolfen chemical park. Hence, there is a complex mixture of chemicals present in the effluents, ranging from personal care products and pharmaceuticals to components used in the chemical industry. In the year 2011 six samples from effluents of the treatment plant were taken over a period of six weeks and investigated on their toxicity regarding different acute and mechanism-specific endpoints. Genotoxicity was detected in the Ames Fluctuation assay, using the Salmonella typhimurium tester strains TA98 and TA100. Estrogenic potentials of the chemicals present in the samples were detected with the human cancer cell line T47D. For the present study the sample showing the highest mutagenic potential in the Ames assay was selected for investigation of the molecular and cellular response in a model organism. In order to identify toxicity pathways in embryos of *Danio rerio* RNA sequencing was used to quantify changes across the entire transcriptome after exposing the embryos to the sample for 120 h. Quantitative real time PCR was used to confirm and quantify changes in gene expression of *Danio rerio* embryos. In addition a variety of genes were selected to investigate mechanisms of response to endocrine disruptors.

WE009

Biological multi-endpoint analysis for an integrative sediment toxicity assessment with higher plants

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In sediment quality assessment molecular methods like transcriptomic analysis offer the chance to bridge the knowlegde gap between cause and effects, and thus link results from analytical chemistry and ecotoxicological bioassays. As gene expression of sediment dwelling organisms is altered directly or indirectly in consequence to sediment toxicity, it links sediment pollution and the caused adverse effects.The aim of this study is to find out whether it is possible to identify molecular biomarkers which can complement and explain those combined results. The main questions of the project are: (i) Do microarray experiments improve the

assessment of sediment associated pollutants with respect to their bioavailability? (ii) Is it possible to differentiate biomarkers of exposure and biomarkers of effect, and thus allow to verify weak adverse effects, as well as to estimate the probability of adverse effects induced by sediment-bound pollutants? (iii) Is it possible to characterize the mode of action of substances which causes adverse macroscopic effects? We developed a new sediment contact assay with the rice plant *Oryza sativa* ssp. *indica*, combined with a subsequent gene expression analysis (DNA-Array und RT-qPCR). Since the genome of the semiaquatic growing rice plant is completely sequenced, it is a suitable testorganism for sediment contact assays and global gene expression studies. For five inorganic pollutants (As (III), Cd (II), Ni (II), Cr (III), Cr (VI)) with high relevance for the sediment macroscopic and molecular endpoints were determined in single substance exposure experiments with spiked sediments. Elongation of root and shoot, were found to be suitable macroscopic endpoints. To distinguish between biomarkers of effect and exposure on the molecular level, the test organism was exposed to concentrations below and above the threshold level for each substance. Suitable test substances and threshold levels were determined in previous range finding experiments. From the data of the global DNA-microarray experiments, differentially expressed genes were chosen as candidate biomarkers of effect and exposure. All candidate genes for biomarkers will be characterized quantitatively on a more detailed time and dose depending scale by means of RT-qPCR.

WE010

Integrative assessment of benzene exposure to Caenorhabditis elegans using toxicogenomics and computational behavior analysis

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Behavior change has been considered an important indicator of chemical toxicity, as it reflects integrated physiological alteration. In this study, we investigated toxic effects of benzene in the nematode *Caenorhabditis elegans* in an integrative manner using toxicogenomics and computational behaviour analysis along with common apical endpoints, such as, survival and reproduction. Benzene exposure led to changes in locomotive behavior and reproduction decline in the *wild-type C. elegans*. Subsequently, microarray followed by pathway analysis revealed 228 genes differentially expressed by benzene exposure (194 up- and 34 down-regulated). To identify genes that regulate behavior response, the most representative three genes were selected from the microarray and pathway analysis as well as worm`s behavior response was monitored using multi-parameters, such as, speed, acceleration, turning rate and meander, on the loss-of-function mutants of those genes. Mutant analysis showed that benzene induced reproduction decline was rescued in *cyp35a2* mutant, whereas it was significantly exacerbated in *pmk-1* mutant comparing with the *wild type*. Multiple behavior parameters indicated that overall activity in *cyp35a2* and *cep-1* mutants was lower whereas the *wildtype* and the *pmk-1* mutant showed higher activity before treatment. After treatment, marked increment of speed and acceleration was observed with *cyp35a2*. Parameters indicating direction changes (meander and turning rate) overall decreased after treatment, especially with *cep-1*. The *wildtype* was only sensitive in turning rate with slight decrease after treatment. Although the *pmk-1* mutant showed no difference in all parameters after treatment, partial body movement was observed showing increase in speed in the head part and decrease in acceleration in the tail part. Self-organizing map revealed that the *pmk-1* mutant group was most clearly clustered and placed in the opposite part of map for the mutant *cyp35a2* across the *wildtype* in the map, indicating that toxicity of benzene would be reversed in two strains. Overall, *cyp35a2* and *pmk-1* genes may play an important role in characterizing benzene-induced alteration on behavior and toxico-physiological responses.

WE011

A comparison of acute stress-responses in Atlantic salmon (Salmo salar) exposed to different oxidation states of antimony using transcriptomics

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There has been increased awareness of potential adverse effects of antimony (Sb) in recipients which may have higher concentrations of Sb mainly from road and tunnel wash runoffs and shooting ranges. Antimony usually appear in the environment as two main oxidation states, Sb (III) and Sb (V). A number of studies stated the general view that Sb(III) may be more toxic than Sb (V), others reported the complete opposite. In addition, the toxic effects of both oxidation states of Sb are poorly documented for aquatic species such as fish. The present study aimed to use transctional changes to study the stress-responses in Atlantic salmon (*salmo salar*) exposed to different oxidation states of Sb, to establish concentration-response relationship of fish exposed to different concentrations of Sb(V), to identify potential modes of action (MoAs) of Sb, and with the ultimate goal to perform hazard and risk assessment for Sb. To achieve these, juvenile Atlantic salmon were exposed 5, 50 and 500 µg/L Sb (V) and 50 µg/L Sb (III) for 72 h. After exposure, both water and internal concentrations of Sb in fish were measured. Gill total RNA was isolated for gene expression analysis using a 60k salmonid oligonucleotide microarray. Array results were analyzed with the support from corredponding bioinformatics to obtain differentially expressed genes (DEGs), related Gene Ontology (GO) functions, protein-protein interaction-based gene networks and toxicity pathways (by mapping salmon DEGs to mamlian orthologs). The results from the present study suggested that both 50 µg/L Sb (V) and Sb (III) induced similar responses in fish. When focusing on the apical functional level, no larges differences could be found between either different oxidation states, or different Sb (V) concentrations. A few consistent MoAs across all treatments may be proposed for Sb, such as inducing reactive oxygen species (ROS) and causing cellular oxidative stress, affecting mitochondrial membrane functions and leading to perturbations of mitochondrial osmoregulation and electron transport chain (ETC), initiating programmed cell death and causing organ injury, binding to or indirectly affecting blood cells and lead to cardiovascular dysfunction. Except for 5 µg/L Sb (V), other Sb treatments may also affect the immune system and nuclear receptor signaling and subsequently activate a series of downstream processes.

WE012

Transcriptomics and multiple stress: can gene expression elucidate interactive effects in response to multiple stressors?

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Current research in ecotoxicogenomics has focused on mechanisms of toxicity of a single stressor which is in contrast with ecological reality. Yet, few studies have investigated responses to multiple stressors and even fewer have investigated the potential interactive effects across a broader dataset of stressors. As a result, knowledge of interactive effects in multiple stress response remains limited. Here, we present the results of a large scale study in which we investigated the effects of 48 different binary combinations of stressors on *Daphnia pulex*. We studied the effects of each binary combination of stressors at both the life-history and the molecular level. Life history performance was recorded during standard 21 day life table experiments and whole-genome microarrays were used to assess gene expression during chronic exposure to two stressors in batch experiments. Of all binary combinations, 40% demonstrated interactive effects at the life-history level. The majority of these interactive effects were antagonistic. At the molecular level, we detected interactive effects by analyzing data according to the concept of ANOVA. Within the set of interactive genes, we observed both antagonistic and synergistic effects on gene expression regardless of the effects on life history. However, combinations of stressors that resulted in antagonistic effects at life history level, also demonstrated a large decline in the cost of gene transcription when compared to the costs of gene transcription upon exposure to the stressors separately. Analysis of gene networks revealed a complex regulation of interactive effects at the gene level.

WE013

Increased disease susceptibility of rainbow trout that were fed polyaromatic hydrocarbons is reflected in transcriptional changes of innate immune-relevant genes

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In the lower reaches of urbanized rivers in the Pacific Northwest of America, sediments are contaminated with polycyclic aromatic hydrocarbons (PAH) which subsequently contaminate food webs. Certain stocks of anadromous salmonids feed in these areas for about two months prior to migration into the ocean. Rainbow trout given food containing 400ppm of a PAH mixture for 50 days suffered increased mortality after exposure to the bacterial pathogen *Aeromonas salmonicida*. Microarray analysis of mRNAs in head kidneys from these fish was conducted 2, 4, 10, and 20 days after pathogen challenge. The head kidney contains a complex mixture of cell types in various stages of differentiation, including high numbers of macrophages and lymphocytes. There were 126 statistically significant differences (ANOVA unequal variance, 5% FDR) in gene expression in PAH-fed compared to control-fed fish at 2 days. This declined to 30, 49, and 3 differences at days 4, 10, and 20, respectively. Early up-regulation of the haptoglobin and transferrin genes by about 400% suggested that PAH did not compromise all aspects of the early response after infection. Since fish started dying 4 days after challenge, initial analysis of differential transcription focused on day 2, likely a time critical in

pathogenesis and activation of the innate immune response. Enrichment of significant Gene Ontology biological processes impacted by PAH exposure was calculated by Fisher's exact test ($p< 0.05$). PAH down-regulated expression of several genes in the pathway for phagocytic cell recognition and lysis of bacteria: (e.g., pentraxin, lysozyme, complement component C6, and C5a receptor). Gene expression associated with phagocyte intracellular killing (cathepsin) was also down-regulated by PAH. Differential transcription provided insight into increased pathogenesis and innate immune system pathways disrupted by PAH in the various cells that populate the head kidney.

WE014

Impact assessment of agricultural inputs into a Mediterranean coastal lagoon (Mar Menor, SE Spain) on transplanted clams (Ruditapes decussatus) by targeted metabolomics

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The Mar Menor lagoon is a shallow coastal basin that receives a wide variety of chemical pollutants associated with anthropogenic activities and it is connected with the Mediterranean Sea mainly through three channels. This lagoon receives water run-off from an intensive agricultural activity which has taken place since 1979. In a previous study the effect of this input on the water quality of the Mar Menor was assessed by means of the measurement of specific biomarkers responses such as the activity of acetylcolinesterase or antioxidant enzyme levels to test neurotoxicity and oxidative stress respectively in transplanted *Ruditapes decussatus* clams (Campillo et al. 2013, Aquatic Toxicol, 142-143: 365-379). This traditional toxicological work with biomarkers has been complemented by the metabolomic profiling of these transplanted organisms for the detection of metabolic biomarkers induced by the agricultural and/or urban pollutants. During the transplantation period a continuous input of PAHs and pesticides through El Albujón watercourse to Mar Menor lagoon was detected, with the greatest input flux corresponding to the organophosphate chlorpyrifos, followed by pendimethalin and naphthalene, and at lower levels acenaphthene, terbuthylazine-desethyl and chlorpyrifos-methyl. PCA of the intracellular metabolites from digestive gland of clams after 7 and 21 days of transplantation showed a different profile of metabolite levels between organisms collected from control and exposed sites. At short exposure time (7 days), there was a remarkable increase of several metabolites in impacted sites compared with control sites, whereas metabolic profiling at 21 days showed that those metabolites were drastically diminished even with lower levels than control sites. These metabolites included 13 from the 21 proteogenic amino acids, osmotic protectants as GBB, and nucleotides such as ITP. This two-phase pattern could highlight a more complicated metabolite response to pollutants than classical biomarkers. Surprisingly, the antioxidant glutathione was maintained constant in the digestive gland of organisms from control and exposed sites.

WE015

A mixed-mode LC-MS method for endogenous metabolite detection

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Traditionally, the analysis of the metabolome is done using LC-MS based on a single separation mechanisms using either reversed-phase (RP), HILIC or ion exchange material. Each method reported has been optimized to yield optimal separation of target metabolites in as short a time as possible, preventing co-elution and ion suppression in the ion source of the MS. One single chromatographic step, however, cannot separate all metabolites. For instance, when using RP chromatography, lipophilic compounds can easily be separated, while hydrophilic compounds elute at the front. The opposite is true for a HILIC-based separation which elutes lipophilic compounds at the front. Furthermore, RP does not separate ionic compounds, unless they have a large lipophilic part, HILIC retention times for ionic compounds are difficult to predict, and ion exchange cannot separate uncharged hydrophilic metabolites. Since a metabolome comprises compounds of all classes (hydrophobics, non-ionic hydrophilics, anions and cations), separation of all metabolites cannot be achieved using single mode chromatography. We have developed a HPLC method that combines all the mentioned separation modes preferentially in a single run to reliably detect and identify the highest number of metabolites. A commercially available multi-mode column (C_{18}, WAX, SCX) was used to perform HILIC, RP, anion exchange and cation exchange chromatography coupled to a triple quadruple MS. Eluents and gradients were optimized to distribute all compound classes over the whole chromatogram. Dozens of endogenous metabolites representing diverse compound classes were separated and serve as base data set to predict retention times of all other metabolites in a database.

WE016

Automatic fitting of concentration-response models and prediction of mixture effects on DNA transcription

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Toxicogenomics is a relatively newly used approach in ecotoxicology, which allows to test effects of toxic substances on gene transcription. Using DNA-microarray method, effects on thousands of genes can be observed simultaneously. Therefore good data analysis and statistical approaches are necessary. The most common data analysis methods (ANOVA and heat-maps) may be insufficient for the deep description of the effects and for further research. In the present work the tool for automatic selection of best-fit concentration-response models for effects of toxic compounds on DNA transcription was developed in R-software. This tool automatically runs through the whole DNA-microarray dataset, fits various types of concentration-response regression models (linear, log-linear, exponential, sigmoid and U-shaped) to each observed effect and finally selects those, which fit the best. This tool was used to analyze DNA-microarray of fish embryo (zebrafish) affected by various single substances (e.g. tetrachloroethylene). The outputs of developed tool (best-fit concentration-response models) can be further used to study the mechanisms of action of toxic compounds or to find the groups of similarly responding genes (for more detailed laboratory testing). The parameters of best-fit models are further used in present work to predict mixture toxicity of substances and to propose a good design of laboratory test of effects of mixtures on DNA transcription to compare predictions with real measured effects (to see if the general mixture toxicity models - concentration addition and independent action - may be applicable to effects on DNA). [Supported by DBU Scholarships for young scientists and by the project CETOCOEN (no. CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.]

WE017

DE-NOVO ASSEMBLY OF THE DAPHNIA MAGNA TRANSCRIPTOME AND DEVELOPMENT OF A GENE EXPRESSION MICROARRAY

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(Eco)toxicogenomics has rapidly evolved as a suite of molecular tools to determine an organism's response to different environmental stressors and to predict toxicological effects in target organisms after exposure to priority chemicals and chemicals of emerging concern. Although "omics" platforms are potentially powerful and robust screening tools for facilitating an in-depth understanding of the modes of action of toxicants, full application of such tools are often limited by lack of both analytical and well-developed bioinformatics tools in non-mammalian organisms such as invertebrates. The aims of the present work were to characterize the *D. magna* transcriptome and develop a gene expression (oligonucleotide) microarray for use in ecotoxicological studies. De-novo assembly of the *D. magna* transcriptome was performed on basis of Illumina Hiseq 2000 RNA-sequencing of RNA extracted from *D. magna* exposed to a range of environmental stressors and pollutants. The de-novo *assembly* provided 46k consensus sequences, represented by 20k clusters and 26k singletons. As many as 35k sequences were successfully annotated to publically available databases (nr, nt, SP, KEGG, COG, GO), whereof approximately 90% were provided by sequence similarities to *Daphnia Pulex*. The protein coding region and resulting amino acid sequences were successfully predicted for the majority of the sequences annotated. A 60k custom Agilent microarray and supporting bioinformatics tools were developed to allow rapid analysis of gene expression profiles in *D. magna* exposed to various stressors. *Acknowledgement* - The authors thank financial support from the Strategic Institutional Initiatives programme (SIS) for Compounds of Emerging Concern to the project MolPoP (www.niva.no/molpop).

Soil Biodiversity and Ecotoxicology (P)

WE018

Testing indicators for soil biodiversity and ecological function in the European FP7 project EcoFINDERS

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One of the aims of 'EcoFINDERS' is the design of policy-relevant and cost-effective indicators for monitoring soil biodiversity to aid the implementation of European soil policy. To that end we generated a list of 19 potential indicators (selected using a logical sieve exercise) that covered a range of methods including both traditional (i.e. taxonomic identification, soil respiration, nitrification) and developing (i.e. metabarcoding , functional gene analysis) indicators from both faunal (from earthworms to protozoa), microbiological (i.e. PLFA, TRFLP) and functional (i.e. water infiltration, suppressiveness, bait-lamina) groups. The purpose of the field sampling was to determine the sensitivity of the indicators to land use change and, secondarily, to evaluate the cost-effectiveness of the individual methods. The indicators are targeted to diversity or ecosystem function.

Where possible we made use of existing long-term sites that would have background and baseline data that would allow calibration of the EcoFINDERS results. To ensure European coverage and applicability we selected agricultural (arable) sites from Atlantic, Continental, Mediterranean and Pannonian climatic zones, along with Atlantic and Continental grassland sites. At each site there were three replicated plots of two contrasting treatments. One of the land uses was considered a control, which had baseline data available, against which the contrasting land use would be compared. The comparisons included: tillage vs. reduced-tillage; cereal vs. fallow; conventional vs. organic arable management; and intensive vs. extensive grassland management. The first sampling at all six sites took place in autumn 2012 and a second round of sampling was completed in spring and autumn 2013. Details of the sampling effort required for each indicator is already revealing some interesting observations. We will present results on: the strategy for sampling; indicator data related to differences between sites and sensitivity to the land-use changes; and initial results on the cost-effectiveness of the indicators.

WE019

Occurrence and distribution of soil microarthropods in agricultural landscapes across Europe with regard to testing for responses to plant protection products

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Within the framework for the authorisation of plant protection products (PPPs) potential risks to soil organisms need to be addressed. Litterbag tests, although the only validated tests available to refine potential risks to the soil decomposer community (other than earthworms), have lately been questioned. As a potential, yet unvalidated alternative, field tests with soil microarthropods (soil mites and collembola) are being conducted in different countries across Europe. While there is a test guideline available for the earthworm field test (ISO 11268-3, 1999) no such document is available for field tests with soil microarthropods. Test protocols for higher tier risk assessment with soil microarthropods are therefore currently being written on a case by case basis. In addition to a lack of guidance for field tests with soil microarthropods there is also a lack of knowledge on typical abundance ranges and species composition of collembola and soil mites in agro-ecosystems. To fill this gap, data on soil microarthropods originating from field studies carried out by European Crop Protection Association (ECPA) member companies in the context of the registration of PPPs were analyzed. Data were used from untreated plots on 40 agricultural sites (36 arable sites and 4 grassland sites) across Europe (22 sites in Germany, 2 in Denmark: 4 in France and 12 in Spain). The studies were done between 2001 and 2013. Due to the lack of guidance the data set is heterogeneous regarding sampling techniques, sampling patterns and study design. Methods used include sampling of soil cores with subsequent extraction of microarthropods (39 sites) and/or sampling with pitfall traps (3 sites). Microarthropods were evaluated at one to seven sampling dates per study and the number of replicate samples ranged from 10 to 180 per sampling date. The level of detail of taxonomic determination ranged from differentiation into the higher level taxa Acari/Collembola only (21 sites), to differentiation to species level for individual groups (mainly Collembola and sometimes Oribatida). The evaluation of the study data provided by ECPA member companies will be complemented by analysis of published data from the scientific literature to give an overview on soil microarthropod populations of agricultural sites (arable vs. grassland) across Europe (abundance, species composition, distribution of life-forms of collembola, identification of typical communities and of dominant species).

WE020

Is general microbial diversity a sensitive indicator of changes in soil function and processes?

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Soil quality has often been considered an indicator of sustainable land management, as it represents the capacity of the soil *to sustain biological productivity and promote plant and animal health,* as defined by The Soil Science Society of America (1997). Microbial communities play a central role in maintaining soil processes, which ultimately impact soil quality and ecosystem functioning. Microbial diversity has been adopted as soil quality indicator to predict the impact of environmental and anthropogenic disturbances, such as agricultural exploitation and chemical contaminations. The addition of biomass-derived charred materials (biochars) is becoming increasingly popular for contaminants remediation and in agricultural application, due to their carbon storage capacity and their ability to increase soil fertility and hence ecosystem services. Several studies have reported that biochar amendment induced a pronounced effect on microbial community structure, and that the induced bacterial communities played an influential role in explaining the enhanced soil fertility. Our previous studies, using microbial fingerprinting methodology (denaturing gradient gel electrophoresis), reported the absence of strong activated carbon effects (a similar charred carbonaceous material) on microbial community compositions in sorbent-based remediation field trials of Norwegian urban soils, impacted by polycyclic aromatic hydrocarbons. These findings contrast with other published reports, but are in line with our present work. We used a next-generation sequencing approach to study the microbial community composition in farmer-led field trials in Zambia, amended with 4 tons/ha biochars, and their respective controls without biochar. Despite an extreme increase (441%) in maize yields recorded in some of the amended soils, we did not observe significant shifts in the microbial community structure from soil samples with and without biochar treatment, and/or stimulation of particular bacterial groups beneficial to plant growth. Our studies suggest that soil microbial diversity alone is not a sufficiently sensitive indicator for soil quality and its response to external changes, as it is not always possible to infer loss or gain of soil functions. Therefore, it is essential to account for microbial functions and activities, in order to understand the interaction between microbiota and the environments.

WE021

Isolation, Identification and characterization of soil borne fungi from QU field 2, field 3 and QU farm

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The mycobiota of soil was investigated in ninety soil samples collected from three different localities in Qatar, biology field2, biology field 3 and University farm. Biology field 2 and biology field 3 are located at longitude and latitude 549350.32 M.E and 2866180 M N 3 549812.00 ME and 2806702.93 M N respectively. Qatar University farm is located in Raodat Al Faras, about 60 km north of Doha (49° 25´ N, 20° 51´ E), 14.1 meters above sea level, with an area of 54.3 ha. Soil samples were collected under *Zygophyllum quatarense*, which was dominated in biology field 2 and 3 and from rhizosphere of fig, lemon, and date palm trees in QU farm. Twenty nine species of fungi belong to 9 genera were isolated from Biological field 2 and 3 at Qatar University. Whereas 19 fungal species belonging to 10 genera were isolated from QU farm. The distribution of soil borne was affected by the microclimate, physic-chemical propriety of soil and types of vegetation. The genus of highest incidence and their respective numbers of species was, *Rhizopus* (24 %, 3 species) isolated from biology field 2 and 3. The genus of lowest incidence was (*Aspergillus* (4.5 %, 2 species) islolated from biology field 2 and 3. Whereas, *Aspergillus* was the most common genera(43.78%, 18 species) isolated from QU farm followed by *Rhizopus* (13.4 %, 4 species) and *Trichoderma* (7.7 %, 2 species). Sixteen isolates were shown an antagonistic activity. *Alternaria chlamydospora* showed highest percentage of antagonistic activity against many species. In contrast, introduction of *Rhizopus arrhizus* led to approximately 62.5% reduction in *Alternaria chlamydospra* growth. Moreover, *Penicillium griseofulvum* inhibited the growth *Alternaria chlamydospra* by 10%. Keywords: Soil borne fungi, antagonist, microclimate, biological control

WE022

In situ variability of the bait lamina response: consideration of the soil moisture content factor in improving test readability

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The bait lamina method can be used to evaluate the biological activity of soil organisms in the field. The overall feeding activity of soil invertebrates is assessed by measuring the consumption of organic-based material ("bait") fixed in thin plastic strips inserted into the soil for a specific time period. The method can be used to investigate the potential effects of chemicals on the soil biocenosis. However, fluctuating environmental conditions (e.g. soil moisture content or temperature) may strongly influence the test response and may hamper interpretation of the results or may lead to bias in the conclusion of the study. In the present work, the influence of soil moisture content was evaluated on the bait lamina responses from a field under pesticide exposure. Changes in overall feeding activity of the soil organisms as well as moisture content were measured in an agricultural brown soil (15% clay, 3% humus) with and without application of glyphosate. Four experiments were conducted over two seasons with different exposure times, that started immediately and several weeks after the herbicide application. Results of the bait lamina tests showed a large variability among the different experiments. Integrating the relationship between feeding activity and soil moisture content improves the interpretation of the bait lamina response in order to determine potential effects of glyphosate on the biological activity of soil organisms. Calibrating the response of *in situ* tests as a function of environmental conditions reduces noise in the data and thus improves the ability to discern

anthropogenic effects from environmental conditions.

WE023
Life traits comparison of earthworm cocoons in populations originating from different agricultural practices
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The application of man-made chemicals on conventional agricultural crops has led to chronic contaminations of the soil, thereby impairing its biota such as earthworms (Lumbricidae). Despite the toxicity of some of these molecules, the ubiquitous presence of earthworms, albeit in reduced numbers in long-term pesticide-contaminated fields suggest that adaptative processes can occur. Adaptive changes have indeed been demonstrated in heavy-metals contaminated areas for invertebrate populations such as isopods or aphids. With regard to the beneficial effects of earthworms for the soil, life traits parameters related to cocoon (oothecae) development have a particular ecological relevance as they are directly linked with population fitness. We compared cocoon traits in two populations of *Aporrectodea caliginosa* originating from either a long-term contaminated (conventional wheat/maize field) or an uncontaminated field (organic cereal/alfalfa crop), both with comparable tillage. Cocoons from the conventional field population had significantly longer hatching times and lower hatching rates, hence decreasing the number of juveniles produced. Cocoon weights were higher in the organic population, but juvenile weights were not significantly different. The ratio juvenile weight / cocoon weight was significantly higher ($p < 0.001$, n=108) in the population from the conventional field, possibly indicating a different energetic investment in cocoon formation. Further investigations of cocoons traits such as phenotypic and energetic traits will be pursued providing us insight in the reproductive allocation of the populations.

WE024
Isolation, Identification and characterization of soil borne fungi from Qatar university field 2, field 3 and Qatar University farm
M.Y. Aljassim, Qatar University / Biology and environment sciences; N.M. Al-korbi, Qatar University / Biological and Environmental Sciences; F.A. Al-Naemi, Qatar University / Biology and Environmental Science
Isolation, Identification and characterization of soil borne fungi from Qatar university field 2, field 3 and Qatar University farm The mycobiota of soil was investigated in ninety soil samples collected from three different localities in Qatar, biology field2, biology field 3 and University farm. Biology field 2 and biology field 3 are located at longitude and latitude 549350.32 M.E and 2866180 M N 3 549812.00 ME and 2806702.93 M N respectively. Qatar University farm is located in Raodat Al Faras, about 60 km north of Doha (49° 25´ N, 20° 51´ E), 14.1 meters above sea level, with an area of 54.3 ha. Soil samples were collected under *Zygophyllum quatarense*, which was dominated in biology field 2 and 3 and from rhizosphere of fig, lemon, and date palm trees in QU farm. Twenty nine species of fungi belong to 9 genera were isolated from Biological field 2 and 3 at Qatar University. Whereas 19 fungal species belonging to 10 genera were isolated from QU farm. The distribution of soil borne was affected by the microclimate, physic-chemical propriety of soil and types of vegetation. The genus of highest incidence and their respective numbers of species was, *Rhizopus* (24 %, 3 species) isolated from biology field 2 and 3. The genus of lowest incidence was (*Aspergillus* (4.5 %, 2 species) islolated from biology field 2 and 3. Whereas, *Aspergillus* was the most common genera(43.78%, 18 species) isolated from QU farm followed by *Rhizopus* (13.4 %, 4 species) and *Trichoderma* (7.7 %, 2 species). Sixteen isolates were shown an antagonistic activity. *Alternaria chlamydospora* showed highest percentage of antagonistic activity against many species. In contrast, introduction of *Rhizopus arrhizus* led to approximately 62.5% reduction in *Alternaria chlamydospra* growth. Moreover, *Penicillium griseofulvum* inhibited the growth *Alternaria chlamydospra* by 10%. Keywords: Soil borne fungi, antagonist, microclimate, biological control

WE025
Trophic transfer of soil arsenate and associated toxic effects in a plant-aphid-parasitoid system
H. Mo, Division of Environmental Science and Ecological Engineering; M. Kim, Animal and Plant Quarantine Agency; Y. Kim, Korea University; Y. Lee, Korea Univ; J. Wee, Division of Environmental Science and Ecological Engineering; K. Cho, Korea University / Division of Environmental
Terrestrial toxic effects of soil arsenate were studied using a model system consisting of Capsicum annum, Myzus persicae, Aphidus colemani. We investigated the transfer of arsenic from soil to aphid and toxic effect of elevated arsenic on each trophic level. Test concentrations were determined to have a no effect on health plant growth to remove the effect of poor plant growth on aphid. Artificial soil was treated with arsenate at 0, 2 and 6 mg/kg, then arsenic concentration of soil, plant tissues (root, stem, leaf) aphids were measured to observe the arsenic transfer. Toxic effects of elevated arsenic concentrations on each species were investigated at population level. Physiological and biochemical responses of plant and aphid were observed. In addition, enzyme activities against reactive oxygen species (ROS) induced by arsenic stress were also investigated. Host choice capacity and parasitism success of the parasitoids were examined. The results suggest that arsenic concentration in plant tissues and aphids were elevated with increased concentration of arsenic in soils. Physiological responses of plants were not affected by soil arsenic but there was change of biochemical responses. Decreased fecundity and honeydew excretion of aphids were observed, elevated activity of antioxidant enzymes indicated that aphids received the ROS stress induced by arsenic. Decreased eclosion rate of parasitoids were observed with increased arsenic treatment in soil. The results showed low concentration of arsenic in soil can transfer through food chain and can impact on higher trophic level species.

WE026
Ecotoxicological assessment of a remediation procedure in a hydrocarbon-contaminated soil
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Contamination of soils with hydrocarbons is of major concern due to their known detrimental effects to both humans and environment. Due to the risks posed by these compounds, the development of remediative techniques for hydrocarbon-contaminated soils has become an important issue. Removal of such compounds from soil applying the Biopile methodology, consisting in an in-situ reduction of the concentrations of petroleum constituents through the use of biodegradation,is one of the available approaches. At the same time, monitoring the residual toxicity of the soil at the beginning and at the end of the remediation is recommended in order to assess the success of the procedure and the final quality of the soil. Methodologies for assessing soil contamination had been traditionally focused on physical and chemical analyses. Nowadays, the need of coupling chemical determinations with ecotoxicity bioassays is corroborated by many authors. However, current available ecotoxicity bioassays can cover many effects and involve a great variety of organisms. Therefore, the selection of the best battery of biotests has become essential for a proper risk assessment of contaminated soils. This study aims to evaluate the effectiveness of a remediation process in a soil polluted with hydrocarbons by chemical and ecotoxicological procedures and at the same time to assess the suitability of different bioassays in order to define the best battery of ecotoxicity tests for the risk assessment of hydrocarbon-contaminated soils. To do so, samples from the polluted soil were analyzed before and after treatment. Chemical analyses focused on the quantification of different hydrocarbons whereas the ecotoxicological approach was carried out determining the soil terrestrial ecotoxicity with several tests and determining the toxicity of leachates from the polluted soil to aquatic organisms. Terrestrial tests assessed effects on mortality, reproduction and behavior of both earthworms and springtails whereas aquatic toxicity was evaluated in *Daphnia magna* and *Selenastrum capricornutum*. Results revealed an important decrease in the concentration of hydrocarbons throughout the remediation process that was correlated with a decrease in the toxicity to aquatic and terrestrial organisms although not in the same intensity for all bioassays.

WE027
Evaluating an alternative procedure for the assessment of the avoidance response with Collembolans
J. Bori, Universitat Politecnica de Catalunya UPC / Centre for Research and Innovation in Toxicology CRIT; J. Ribo, Tecnical University of Catalonia UPC / Centre for Research and Innovation in Toxicology CRIT; C. Riva, Universitat Politecnica de Catalunya UPC / Centre for Resarch and Innovation in Toxicology
Hazards of chemicals to soils can be assessed by standardized ecotoxicity methods that use a wide range of soil organisms to evaluate effects on mortality, reproduction or behavior among others. Together with earthworms, springtails are the invertebrate species most frequently used for soil toxicity testing. In relation to sub-lethal assays available for Collembolans, reproduction tests have the main disadvantage of their long duration and high number of organisms required. Behavioral tests, on the other hand, can detect detrimental effects in much shorter time and with simpler procedures. The ISO standard 17512-2 shows a methodology for measuring the avoidance response of Collembolans when exposed to a polluted soil. This methodology has the disadvantage of a high variability of the results usually associated to avoidance tests that cannot always be improved using an artificial standard soil. However, such drawback could be partly overcome by measuring at several time intervals. Given the aforementioned problems, it seems necessary to develop a simplified and miniaturized procedure that a) allows measurements at several exposure times with one single replicate, b) does not need reference soils, c) does not require much test substance and d) does not use many organisms. In this study we present and evaluate an alternative procedure for the assessment of the avoidance response of springtails to chemical contaminants that involve using filter papers as substrate. To do so, avoidance response tests have been carried out following both the standard ISO and our alternative procedure and results have been compared. Selected test substances include commercial pesticides, heavy metals, detergents and hydrocarbons. A secondary objective aimed to study the application of the avoidance test in the assessment of toxicity of wastewater effluents comparing it with assays using daphnia or algae as test organisms. Previous results point out a good correlation between both avoidance tests in the determination of the avoidance response.

WE028
Genetic methods to characterize test species in ecotoxicology:
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Almost as long as earthworms have been used in standard ecotoxicological tests there is a discussion going on whether the compost worms recommended for these tests belong to one species or two. There is plenty of evidence that two taxa, *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Lumbricidae), can be distinguished by morphological, physiological, behavioural and molecular traits. However, it is unclear whether these differences are sufficient to interpret both taxa as separate species. In order to assess the practicability and robustness of the genetic characterization of earthworms used in ecotoxicology, in particular the species *Eisenia fetida* and *Eisenia andrei*, an international ring test was organised. Coded samples of these worms (four individuals per species and laboratory), labelled by each participating laboratory (in total 29, from 16 countries) as *E. fetida*, *E. andrei* or *Eisenia* sp., were sent for DNA extraction, amplification and sequencing to five laboratories located in Belgium, Canada, Germany, and Spain (2). All steps of the preparation of the samples were described by specific Standard Operation Procedures. Data assessment is still under way, but about 40% of all data were evaluated by means of a neighbour-joining analysis based on the uncorrected pairwise distances among all COX1 sequences (581 bp). This analysis revealed three distinct, homogeneous groups: one including *E. andrei* voucher sequences (mean within group distance 0.026) and two with *E. fetida* voucher sequences named *E. fetida* A and *E. fetida* B. The mean within group distances in *E. fetida* A and *E. fetida* B were zero. The mean distance between *E. fetida* A and *E. fetida* B was 0.112, whereas the distance between the former and *E. andrei* was 0.142 and 0.143, respectively. Such divergence levels within the COI region are usually found only between species. The existence of a cryptic species pair within *E. fetida* is therefore a plausible hypothesis in need of further investigation. Remarkably, no individual of the *E. andrei* clade was initially identified as *E. fetida*. However, this was not true the other way round, meaning that *E. fetida* often resembles *E. andrei*. The results of this ring test will be discussed with standardisation organisations (OECD, ISO) in order to clarify how this information on species identification could be used to improve ecotoxicological routine testing.

WE029
Ecotoxicology of soils contaminated with Pb and Zn by soil respiration bioassay
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Pb and Zn are common pollutants in soils related to anthropogenic activities, causing a significant toxicological risk to living organisms and serious degradation of the ecosystem. Soil respiration (Rs) is the second largest flux in the global carbon cycle and is mainly related to soil microbial and biochemical processes. The ability of bacteria to decompose complex substrates is significantly reduced by the presence of metals, so the amount of CO_2 evolved is a very reliable indicator of the effect of pollution, included as endpoint in Ecological Risk Assessments (ERA). Some authors have observed that some soil properties have influence in Rs, like clay content, organic carbon content, pH or carbonates compounds; thus, these parameters ought to be controlled to estimate the metals effects on microbial populations. We studied seven different soils (H1-H7) with contrasting properties and with different pollution levels of Pb (0, 500, 1000, 2000, 4000 y 8000 mg kg^{-1}) and Zn (0, 600, 1200, 3000, 6000, 9000 mg kg^{-1}), to assess the influence of soil properties in the metals contamination and in the inhibition of soil CO_2 flux (Rs). The experiment was carried out with a microbiological analyzer (μ-Trac 4200 SY-LAB). According to Pb pollution results, in all treatments there was a negative correlation between the soluble Pb and the respiration rate. The Rs showed no toxic effect for carbonate (H1, H2, H3) and rich in organic carbon soils (H1, H5). While H4 and H6 soils (sandy texture, with no carbonate and low organic carbon content) evinced significantly lower respiration rates for contamination levels higher than 1000 mg kg^{-1}. H7 (Bt horizon) showed the most sensitivity to Pb contamination, appearing differences in Rs at 500 mg kg^{-1} treatment. Samples contaminated with Zn exhibited similar behaviour to Pb. The carbonate and the rich in organic carbon soils showed no significant reductions to Rs. However the samples most sensitive to Zn contamination were, in this case, H4 and H6 which significantly reduction in CO_2 flux from contamination levels higher than 600 mg kg^{-1}, while H7 showed toxic response above 1200 mg kg^{-1} of Zn added. These results highlight the key role for the toxicity of soils rich in organic carbon and carbonates, even at high levels of potentially toxic elements such as Pb and Zn; therefore soil properties should be considered to develop metal toxicity thresholds to ERA by the use of multiple bioassays application under the same conditions.

WE030
Do traits provide a clue for predicting metal accumulation and sensitivity in different earthworm species?
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There is no clear consensus in the literature on the metal accumulation pattern and sensitivity of different earthworm species. In the present study, accumulation and toxicity of Cu, Cd, Ni, and Zn in the earthworms *Lumbricus rubellus* (epigeic), *Aporrectodea longa* (anecic), and *Eisenia fetida* (ultra-epigeic) were determined after 28 days exposure in two soils. Metal accumulation and sensitivity were interpreted using the specific traits of different earthworm species. Results showed that for all four metals tested *L. rubellus* was the most sensitive species, followed by *A. longa* and *E. fetida*. At the same exposure concentration, internal concentrations followed the order: *L. rubellus* > *E. fetida* > *A. longa* for Cu and Ni, *L. rubellus* ≈ *E. fetida* ≈ *A. longa* for Cd, and *L. rubellus* > *A. longa* > *E. fetida* for Zn. Langmuir isotherms were used to model metal accumulation at both nontoxic and toxic exposure concentrations. The Cu, Cd, and Zn concentrations in *E. fetida* generally leveled off at high exposure concentrations but not for the other two species. *A. longa* showed a high capability of regulating internal Ni concentrations. The traits-based approaches suggested that most likely a group of earthworm traits together determined (differences in) metal accumulation and sensitivity. More research is needed in this respect to build up solid relationships between species-specific responses and traits, enabling cross-species extrapolation of accumulation and toxicity data.

WE031
Development and standardization of an ecotoxicological test method for the risk assessment of GMP
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Before a genetically modified plant (GMP) may be released into the environment and placed on the market in the European Union (EU), an environmental risk assessment (ERA) according to EU directive 2001/18/EC must be performed. Currently, testing of effects of GMP on non-target organisms is based on ecotoxicological test methods developed for the assessment of chemicals. This does not fully comply with directive 2001/18/EC, which demands a case-specific ERA. According to Annex II of the directive a 'case' is defined as a combination of the parent organism, its genetic modification and the possible receiving environment related to the intended release and use of the GMP. As the standard test organisms used for the assessment of chemicals do usually not occur in the receiving environment of GMP, they cannot be considered adequate. According to an ecology-based selection approach test species should be selected from organism groups relevant to the receiving environment and to the various exposure pathways and that cover different taxonomical and physiological groups. Nevertheless, testing of any species in the laboratory also needs to be practical for a standardized application. Hence, the aim of this R&D project (2012-2015) is the development and standardization of a laboratory ecotoxicological test especially for the ERA of GMP. This aim will be reached in three steps. First, the black fungus gnat *Bradysia difformis* Frey (Sciaridae: Diptera) was identified as a test species and its mass rearing in the laboratory was established. The second and currently ongoing work step comprises the actual development and trial of the test method that will meet the above mentioned characteristics of the assessment of GMP. The uptake of different plant materials (oat meal, potato, maize) could be confirmed by visually assessing the gut content of larvae. First results are presented regarding the hatching success of imagines exposed to potential reference substances (positive control) as well as towards GMP (MON810 maize) material. In the third and final work step this method will be described in a draft guideline according to the specifications of the OECD or ISO.

WE032
Joint toxicity of chlorpyrifos and mancozeb to the terrestrial isopod Porcellionides pruinosus: a multiple biomarker approach
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Ferreira, CESAM Universidade de Aveiro / Departamento de Biologia and CESAM; D.N. Nunes Cardoso, CESAM University of Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology
The increasing concerns with the safeguarding of crop productivity have led pesticides to become a critical tool in the modern intensive agriculture regimes worldwide. Albeit this pivotal importance, these compounds are also known to entail deleterious consequences to non-target organisms in agroecosystems. Moreover, given the requisite of acting on different crop pests/diseases, soil-living organisms are often simultaneously exposed to several pesticides. Since mixture effects have been shown not to necessarily reflect the toxicity of its components, the understanding of the mechanisms by which toxicity is induced during multiple exposures becomes critical to predict the possible effects associated to the release of these compounds on the ecosystems. Aiming to contribute for this discussion, in this work we tried to evaluate the age-related differences on the susceptibility of the terrestrial isopod *Porcellionides pruinosus*, when exposed to a mixture of two pesticides: the organophosphate insecticide chlorpyrifos and the dithiocarbamate fungicide mancozeb. In order to have an insight into the several pathways of toxicity prompted by this mixture, a multiple biomarker approach was employed in juveniles and adult organisms, as well as the measurement of energy reserves and the assessment of cellular energy allocation. Results were integrated using the Integrated Biomarker Response model and deviations to the additivity were assessed using the reference model of independent action.

WE033
The comparison of adult and juvenile earthworms Eisenia fetida sensitivity to cadmium
J. Zaltauskaite, Department of Enviromental Sciences
The heavy metals pollution of soils pose serious risk to soil dwelling organisms and earthworms, as soil keystone species, are considered to be one of the most sensitive groups of soil invertebrates. Cadmium is a non-essential, strongly toxic element causing severe biochemical, physiological and reproductive effects in many organisms. During the present study our aim was to compare the adult and juvenile earthworms *Eisenia fetida* sensitivity to cadmium. Adult and juveniles earthworms were exposed to 1-100 μg Cd g^{-1} of soil. Adult worms were exposed to metals for 4 weeks, juveniles – for 14 weeks. The juveniles *E. fetida* were found to be more sensitive to Cd than adults. The risk of death of juveniles increased with Cd concentration in soil, but there was no significant mortality of adults at all tested Cd concentrations. The growth of juveniles (measured as weight) was also more affected than that of adults ($p< 0.05$). The weight of adult earthworms exposed to cadmium tended to show a dose-related decrease though a statistically significant decline was detected only in case of the highest concentration (100 μgCd g^{-1} soil). Cd severely affected the maturation of juvenile at the highest concentrations, worms did not reach sexual maturity at all. In case of adult worms, Cd inhibited the cocoon production rate in range of 18 – 65 %.

WE034
Ecotoxic effects of antibiotics depend on micro-scale variability of soil microbial diversity
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Veterinary antibiotic such as SDZ are applied with manure to agricultural soil. Antimicrobial effects on the soil microbial diversity were reported, but often restricted to homogenized bulk soil. Nonetheless, the assessment of antibiotic fate and effects on soil microorganism in field soil habitats with different physicochemical and biological properties such as rhizosphere soil, earthworm burrows, or soil macroaggregates are almost unknown. To evaluate these aspects, laboratory and field experiments were conducted using different soil habitats of a Luvisol. We used microbial enzyme activity, phenotypic (PLFA) and genotypic (16S rRNA gene fragments of *Pseudomonas* and *β-Proteobacteria*) characteristics to evaluate the impact of SDZ on soil microbial communities in different soil microhabitats. Data were evaluated by principle component analyses (PCA) and two-way ANOVA with post-hoc tests. The microbial status varied with the habitat and often was interacting with the treatment. The SDZ distribution and microbial responses to the pollutant were largely different compared to bulk soil at earthworm burrow and soil macroaggregate level. SDZ-derived effects in rhizosphere soil differed from that in bulk soil despite a largely unaffected SDZ distribution. Some of these microbial responses to SDZ were similar in laboratory and under field relevant conditions.

WE035
Vicia-micronucleus test to assess soil genotoxicity potential
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The assessment of the environmental impact by anthropological activities is an important challenge for the 21st century. Since the industrial revolution, the contamination of water, soil and air by heavy metals or organic pollutants indeed speeded up (Jones and De Voogt, 1999; Weiss et *al.,* 1999; Han et *al.,* 2002). Although chemical analyses reveal the typology of pollution in a given matrix, they do not give information about the real ecotoxic potential of the matrix, which takes into account the bioavailability of pollutants (Vasquez and Fatta-Kassinos, 2013). This information requires the development of biological tests, and especially with plants. Plants present indeed a particular interest in ecotoxicology due to their (i) immobility, (ii) important roots network, and (iii) fundamental role in ecosystems as primary producers (Hock and Elstner, 2005). Ma (1999) described higher plants as the most sensitive organisms for the detection of mutagens and genotoxic effects of environmental pollutants. Although ecologically relevant for soil toxicity assessment, plants are surprisingly not the most commonly used organisms for genotoxicity tests (Grant, 1994; White and Claxton, 2004). Genotoxicity - simply defined as the toxicity on the genome - is an indicator of dysfunctions appearing at sub-lethal concentrations. An easy endpoint to observe is the formation of micronuclei, that are small nuclei appearing whenever a chromosome fragment or a complete chromosome is not incorporated into the nuclei during mitosis (Rieger et al., 1968). It therefore reveals a break of genetic material (clastogenic effect) or a dysfunction of mitotic spindles (aneugenic effect). The aim of this study was to assess the genotoxic potential of six soils contaminated by heavy metals, in the context of phytoremediation by woody species. The recently standardized *Vicia*-micronucleus test (ISO 29200) was performed with a direct exposure approach. Results showed that four soils induced genotoxicity revealed by an increase of micronuclei frequency. These data will be discussed with heavy metal analyses.

WE036
A novel passive dosing-based PICT detection method reveals no increased tolerance to benzene, toluene, ethylbenzene and xylene (BTEX) in soil bacterial communities exposed to gasoline vapours
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The pollution-induced community tolerance (PICT) approach is used to reveal toxicant-induced adaptation in biotic communities. An uneven species-sensitivity distribution is generally considered as a prerequisite for PICT development, and it may therefore be questioned whether toxicants with non-specific modes of action (e.g. BTEX compounds showing general narcosis) are able to generate PICT responses. However, some specialized groups of bacteria possess several specific resistance mechanisms (e.g. extrusion pumps) for BTEX compounds suggesting that there should be a potential for PICT development. We here provide the first PICT study of soil bacterial communities exposed to various doses of gasoline vapours using a novel via-headspace passive dosing technology to ensure defined toxicant exposure during both PICT selection and PICT detection phases. PICT selection was carried out in sealed, yet oxic, soil microcosms (sandy loam, pH 6.8) exposed to gasoline vapours for a period of 6 weeks under controlled laboratory conditions (15 °C, dark). Inserts containing gasoline mixed in various proportions with miglyol oil were placed inside microcosms. The passive dosing approach ensured reproducible exposure as confirmed by gas chromatography-mass spectrometry analysis of carbon disulphide extracted soil samples taken during the PICT selection phase. Bacterial communities were extracted from soil microcosms after 6 weeks and PICT to gasoline and single BTEX compounds were investigated using a passive dosing [^{3}H]leucine incorporation approach. Bacterial growth activity ([^{3}H]leucine incorporation rate) and cumulated soil microbial activity (soil respiration) were measured throughout the PICT selection phase. Low gasoline exposure dramatically increased both soil bacterial growth and soil respiration rates, whereas high gasoline exposure strongly inhibited both microbial activities relative to unexposed control soil. This indicates that volatile gasoline compounds acted as both growth substrates and toxicants, respectively. Remarkably, bacteria extracted from gasoline exposed soils for 6 weeks did not show increased tolerance neither to gasoline vapours nor to single BTEX compounds. The lacking potential for community adaptation is consistent with the observed toxic effects on microbial activities (i.e. no functional redundancy following toxicant exposure) and may compromise microbial community functioning (e.g. biodegradation) in severely gasoline contaminated soils.

WE037
Molluscidal baits represent high risk to key species in the soil compartment
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The application of ready to use molluscicide baits on soil surface is a common practice to control terrestrial gastropods. The ecotoxicological evaluation of these baits has been conducted mainly to non-target soil organisms such as earthworms, however, there seems to be a gap concerning the evaluation of the effects that the application of these baits could pose to soil arthropods. According to the Draft Assessment Report (DAR) for metaldehyde and methiocarb, testing using arthropods, such as collembolans and isopods, are exempted because no effects were observed in earthworms exposed under realistic levels of those compounds. In this work the ecotoxicological effects of molluscicide baits to the collembolan *Folsomia candida* were evaluated, and results were then confronted with the ones already published with the isopod *Porcellionides pruinosus*. Molluscicide baits containing metaldehyde and methiocarb were applied on the soil surface and effects on survival, reproduction and avoidance behaviour in this collembolan species were assessed. Regarding this objective, two exposures of 28 days (according to the ISO 1999) were performed. In the first one, molluscicidal baits were provided only at the beginning of the exposure, and on the second one, baits were replaced for new ones after 14-days of exposure, to simulate the recommended application rate provided by the manufacturer. In the avoidance test, significant differences were observed only for methiocarb, and for all treatments collembolan showed a preference for the contaminated side. In the single bait application exposures, metaldehyde showed a significant increase in mortality and a significant reduction of the number of juveniles. For methiocarb, significant differences in the number of juveniles was observed, but no difference was found on survival. In the experimental set up where a 2nd set of baits were applied, the toxic effects after 28 days of both chemicals was higher than in the previous test, with significant differences at all the treatments of both reproduction and mortality. Analysing mortality and different biomarkers activity, both molluscicides caused severe effects to the terrestrial isopod *P. pruinosus* in short-term exposures. These results are in accordance with the ones obtained for the collembolan *F. candida*, highlighting the fact that molluscicide application and formulation should be revised as they represent a high risk to non-target soil invertebrates.

WE038
The effects of mercury contaminated food to Folsomia candida: bioaccumulation and growth
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Mercury (Hg) pollution is a worldwide problem that can pose a serious threat not only to ecosystems, but also to humans, due to their biomagnification along food chains. Despite growing concerns about the potential adverse effects of elevated mercury concentrations within the environment, few toxicity data is available for soil invertebrates. Current recommended ecotoxicological tests with the springtail *Folsomia candida* provide useful data regarding their reproduction output, upon a soil exposure of 28 days (ISO, 1999). It is widely known that the toxicity of chemicals for that collembolan species comes mainly from the interstitial water located in the soil pores, but the evaluation of other exposure routes and endpoints using short-term periods can provide additional information on xenobiotics' effects to sentinel species. In the life trait of any species, growth is of most importance for the continuity of any species, as they are closely related to the organism reproduction and therefore to population sustainability. In addition, the exposure through food can also provide new insight on toxicity effects to soil invertebrates as their main role in the ecosystem is related with decomposition processes.
Organisms from the species *F. candida* were exposed on plaster of Paris/charcoal substrate to Hg contaminated food at different concentrations (1 to 4 ppm) during a complete life-cycle. During this exposure period collembolan growth was recorded every two days, and a Von Bertalanffy's growth curve derived along with growth rate. Regarding that collembolans are known to avoid contaminated food and/or soil, another aim of this study was to infer if differences in growing rates would result from food avoidance or Hg toxicity. For that, a bioaccumulation test was also carried out and the quantification of Hg in the organisms and the uptake and assimilation rate constants calculated. The results obtained provided not only information for the implementation of future growth tests as complement to the actual toxicity tests, but also new information on Hg effects on growth and bioaccumulation and elimination rates.

WE039
Assessing the potential hazard of wastes as soil amendments
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The growth of the world population, and consequencially the development of agricultural and industrial activities have increased the production of wastes.The deposition of these wastes in landfills is of high environmental concern and alternatives must be found. The use of wastes as soil amendments in agriculture could be an important alternative due to their high nutrient and organic matter contents and also by increasing agreggation and structural stability of soils increasing water infiltration. However this practice should be carefully monitored due to the presence of metals and organic chemicals with hazardous effects on soil organisms. In this study a range of ecotoxicological tests was performed to assess the potential hazard of these matrices towards soil fauna and plants. To attain this goal reproduction tests with *Folsomia candida*, *Enchytraeus crypticus*, *Hypoaspis aculeifer*, mortality and reproduction tests with *Eisenia fetida* and germination tests with *Lactuca sativa* and *Lolium multiflorum* were performed for 9 wastes with different origins (industrial, urban and agricultural). For each test battery EC50 values were estimated for each test organism. Tests performed showed that the least toxic wastes were in general composted wastes such as the MMSWC (Mixed municipal solid wastes compost) and the AWC (Agricultural wastes compost) which produced no effects towards mites at the highest tested concentration. The most toxic wastes were those with high pH values (pH>8) such as the AIS (agro-industrial sludge), MSS (Municipal slaughterhouse sludge), PMW (pulp- and paper-mill waste) wastes. However for the AIS waste although presenting a high toxicity for soil fauna (ex: *Hypoaspis aculeifer* EC50= 1.45%) it had a comparatively low phytotoxicity (ex: *Lactuca sativa* EC50= 31.7%). Overall the results obtained showed that although mites were generally less sensitive and collembolans the most sensitive species, the species tested had variable sensitivities towards the different wastes. This variable response towards wastes demonstrates the need of using a large test battery in assessing their ecological risk.

WE040
Is the sensitivity of Folsomia candida from laboratory cultures comparable to that of field organisms?
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The toxicity of chemical compounds in soil is often evaluated through laboratory reproduction tests following ISO guidelines. The springtail *Folsomia candida* is a commonly used species in ecotoxicological soil testing. This species reproduces parthenogenetically and the use of different clonal lineages may interfere in the sensitivity of these organisms towards chemical compounds. Specimens taken from laboratory cultures may represent clonal lineages that are not representative of clones present in the field, which may compromise the ecological relevance and the reliability of results obtained in laboratory tests for ecotoxicity evaluations. Aiming to evaluate the comparability of the sensitivity of organisms from laboratory cultures of *F. candida* of the University of Coimbra, reproduction tests with springtails from laboratory and field cultures were performed using a gradient of copper spiked soils. A natural agricultural soil was used as test substrate and individuals of *F. candida* from the same area where the soil was collected were used as test organisms. The reproduction tests performed showed similar toxicity towards copper for springtails independently on their source. The EC50 values estimated for laboratory and field springtails were 543 (151-937) mg Cu/kg and 557 (150-964) mg Cu/kg, respectively. These results lead to assume that the sensitivity of springtails from laboratory cultures is comparable to that of springtails from the field. However, tests using organic compounds like pesticides as test substances should be performed to confirm the reliability of using springtails from laboratory cultures, as representative of field populations.

WE041
A rose by any other name would smell as sweet: The "namesake" obstacle to overcome when immediate analysis is required in non-radiolabelled Eisenia fetida bio-concentration studies.
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Whilst uncommon, it is sometimes necessary to undertake immediate analysis of soil and earthworms during laboratory earthworm bio-concentration studies. Aside from logistical challenges, the primary obstacle to overcome was found to be the namesake of the test organism, *Eisenia fetida*, the fetid yellow secretion known as vermiwash. During the development of a non-radiolabelled analytical method for an earthworm bio-concentration study where immediate sampling was necessary due to frozen storage instability, the obstacle of the vermiwash produced by the earthworms was overcome. Several rapid preparation techniques were trialled; each approach resulting in differing volumes and properties of vermiwash. Several extraction systems were also trialled before a suitable method of preparation and extraction technique was developed to overcome the primary obstacle: the namesake of *Eisenia fetida*, vermiwash. Preparation and extraction techniques are compared and a conclusion presented on the adopted procedure in this case.

WE042

Evaluation of terrestrial ecotoxicity of ionic liquids - a neglected issue

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During the recent decades, ionic liquids (ILs) have generated a lot of interest as a new promising group of compounds. They are composed entirely of ions, liquid in a broad temperature range and practically non-volatile. The number of combinations of ions that form ILs is estimated to be practically infinite, so they can be custom produced in order to suite a desired application. They have a widely spread use, mostly as a reaction medium in numerous chemical transformations, separations and extractions that until recently could only be carried out in toxic organic solvents. ILs are usually described as "green", but they are chemical products, and as such have to fulfill the European Community regulation on chemicals and their safe use called REACH. Due to their immeasurably low vapor pressure ILs cannot be found in the atmosphere, but their water solubility is often high and they are stable, so they can end up in industrial and laboratory effluents and consequently cause water and soil contamination. The analyzed ILs include representatives of a new family of protic ILs based on polysubstituted amines and organic anions, and some of the most frequently used imidazolium and pyridinium based aprotic ILs. The methodology was based on OECD and ISO guidelines. Comparing the ecotoxicity results for the representatives of these two groups, it can be seen that not all of the analyzed ILs have the expected "green" profile. The presented results for the terrestrial ecotoxicity (seedling growth inhibition test, and carbon and nitrogen mineralization) are a novelty, especially those concerning the effects of ILs on carbon transforming and nitrifying microbiota, because the usual focus in the literature has almost exclusively been on the aquatic ecotoxicity of the ILs. The protic ILs proved to be non-toxic in most of the performed tests, having the EC50 values up to several orders of magnitude lower than the EC50 for the aprotic ILs. The analyzed protic ILs are potentially biodegradable in soil, unlike the aprotic ILs. All of these findings also indicate that the ILs with simpler and lineal structure could be considered as environmentally safer than the ILs predominately used up to date, which have bulky organic anions with long alkyl chain substituents. The effects on ILs on soil microbiota have to be studied in more depth in order to have a complete picture of the potential negative environmental impact of ILs.

WE043

Flow cytometric analysis of soil algal toxicity in soil extracts polluted by heavy metals

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Soil algae are major producers in terrestrial ecosystem. They play an important role in trophic levels in environment. Heavy metal-polluted environment induced adverse effects of ecosystem. In this study, we assessed toxicity of heavy metal-polluted soil extracts on soil alga *Chlorococcum infusionum*. *Chlorococcum infusionum* of exponentially growing culture was used in the toxicity test. Test chemical was copper and nickel as primary soil pollutants, and soil extracts were extracted from copper-polluted OECD artificial soil. Flow cytometric analysis was performed after 6 days of treatment. Cell size and granularity was measured by forward scatter channel (FSC) and side scatter channel (SSC). Autofluorescence of native pigments was detected by FL3 filter. As a result, intensities of FSC, SSC, and FL3 filter were reduced as a function of exposure concentrations of copper and nickel. It indicates that copper and nickel caused decreases of cell size, granularity and autofluorescence of native pigments for *Chlorococcum infusionum*. *This subject is supported by Korea Ministry of Environment as the GAIA project (2012000540011).*

WE044

Optimization and validation of oxidative stress and metallothionein markers in Folsomia candida, a case study for Cu and Cd

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Folsomia candida (Collembola) is a standard ecotoxicological species; assessment includes organism endpoints, namely survival and reproduction. Here, for the first time a battery of biomarkers to assess oxidative stress was optimized and validated. The antioxidant capacity was measured by the activities of catalase (CAT), glutathione reductase (GR), glutathione-s-transferase (GST), and levels of total glutathione (TG). Moreover, metallothioneins (MT) were also quantified. The oxidative damage in the lipid membranes was estimated by lipid peroxidation (LPO). The exposure included the essential and non-essential metals Cu and Cd, using a series of sampling times along a 10 day period (0, 2, 4, 6 and 10 days), in LUFA 2.2 natural standard soil. Exposure concentrations were selected based on their reproduction EC50 values, 1000 and 60 mg/Kg d.w. soil, for Cu and Cd respectively. Results showed that although both metals caused an increase in the MT levels after 4 days, Cd seemed to act as a stronger oxidant agent compared to Cu and consequently more damaging: Cd mobilized/activated more antioxidant enzymes, but the increased activities were not enough to prevent LPO. This study confirms the oxidative stress caused by both metals, and that this occurs to different extents [despite the use of same reproduction EC50] indicating that toxicity must be more irreversible for Cd than for Cu. Data shows that oxidative stress biomarkers can be successfully used in the standard soil species *F. candida* and the usefulness of complementing information to a more mechanistic level. The selected sampling times gave a good indication of the markers dynamic and can be reduced/adapted in future testing.

WE045

Different food sources make different in reproduction and body weight of Eisenia fetida

M.H. Haeba, Benghazi University / Zoology; A. Mohamed, Benghazi University

Feeding is very critical parameter to be considered in ecotoxicology test. Different food source can alterate the effect of toxic on the organisms and change the result of the test. Our study were conducted to show the effect of different five food source (alfalfa, barley, corn, bread, manure and mixture of food) on the adult earthworm with clear clitellum. The study were run for a month on laboratory conditions. Growth (body weight) and reproduction (cocoon numer) were evluated. During the peroid of the study ten earthworm were cultured in 250g of artificial soil with three replicates. Five gram of food were added once a week. Means of body weight of earthworms feed on alfalfa, barley, corn, bread, manure and mixture of food were 5.86±.3, 6.25± 0.3, 6.3 ± 0.3, 6.66±1.16, 4.93±0.47 and 5.93±1.02 respectively. Means of cocoon number of earthworms feed on alfalfa, barley, corn, bread, manure and mixture of food were 66±21, 109±30, 95±14.6, 105±7, 69±6.5 and 122±7.76 respectively. The result shown significant different in body weight between some groups eg. Alfalfa and manure, barley and manure groups as well as corn and manure groups $P < 0.05$. However, the highest number of cocoon were recorded in mixture group and the lowest number were in the alfalfa group. Significant different were shown between both of (alfalfa, corn and manure groups) and mixture group $P < 0.05$. Our result recommend to feed *Eisenia fetida* on the mixture of food and put more emphasis on the food types important during the culture as well as in the experiments.

WE046

Avoidance behaviour of Eisenia fetida to contaminated soil around Benghazi city, Libya

M.H. Haeba, Benghazi University / Zoology; H. Elwerfalli, Benghazi University; W.A. Awgie, Benghazi University / Zoology

Earthworm *Eisenia fetida* able to avoid contaminated soils, avoidance tests has been developed and validated for some years. It has a great potential as early screening tools. This rapid response enhances the utility of the test to evaluate contaminated sites. It has been indicated that earthworm avoidance behaviour is an ecologically relevant parameter for assessing harmfulness of field-contaminated soils. The objective of this study to evaluate soil contamination around Benghazi city by using avoidance test as rapid, inexpensive, and easy tools. Series percentage (100, 75, 50, 25%) of soil which collected from locations around Benghazi city (Bouatni, Hawari, Lowifia, and Jarotha) which used in avoidance test under control conditions for 24 hours. The locations soil was mixed with artificial soil to get the desired percentages. Earthworm completely avoid all soil 100% from all locations as well as Bouatni soil 75%. However, Hawari, Lowifia, and Jarotha soil 75% were avoided by means 6±1.2, 6±3.6, 9±1.2 respectively. At 50% soil in all locations were no avoid behaviour accept that in Bouatni soil 50% were avoided by 6±3.6. Earthworm expose to concentration below these mentioned above showed no avoid behaviour. The outcomes from avoidance behaviour tests might bring rapid information for future decisions on the evaluation procedure of contaminant sites, terrestrial risk assessment and soil quality criteria studies.

WE047

Using earthworm to investigate soil contamination in different time of exposure around Benghazi City, Libya

M.H. Haeba, Benghazi University / Zoology; H. Elwerfalli, Benghazi University

Pollution of terrestrial ecosystem is a serious environmental problem worldwide. Earthworm is considered as a domain soil organism. It has been recommended test species to evaluate soil contaminations in acute as well as chronic toxicity. Earthworm density and biomass are strongly influence by pollution. In this study, mortality, biomass, cocoon number of *Eisenia fetida* were examined during 45 and 75 days to series percentage (100, 75, 50, 25%) of soil which collected from locations around Benghazi city (Bouatni, Hawari, Lowifia, and Jarotha) under control conditions. The locations soil was mixed with artificial soil to get the desired percentages. The highest mortality was in Bouatni soil 100%. However, no mortality were recorded more than 10% elsewhere after 45 days exposure. After 75 days exposure 50% of death were in Hawari soil 100%. Otherwise, no mortality were in other treatment soil more than 10%. Cocoon numbers were decreased significantly in all locations at 100, 75, 50% as well as in Hawari soil 25% after 45 days exposure. Cocoon number were decreased after 75 days of exposure

significantly in all locations Bouatni, Hawari, Lowifia, and Jarotha 100% as well as in Bouatni and Jarotha soil 75%. Earthworms body weight were decreased significantly $P < 0.05$ in locations soil (100%, Hawari, Lowifia, and Jarotha) compared to control earthworm after 45 day. However, after 75 days of exposure only significant decrease in body weight were in Hawari 100% compared to control. Our results shown different time of exposure change the result dramatically. Decreased in body weight as well as cocoon number which can lead to decline in earthworm populations and consequence to reduce soil fertility. This study was to investigate contamination soils around Benghazi city by using biota as well as put more emphasis on using earthworm as bioindicator.

WE048

Effects of Zn, Cd and Ni on the midgut structure of the cricket Gryllus assimilis

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The aim of the study was to examine changes in the structure of midgut of *Gryllus assimilis* fed with media contaminated with Zn, Cd or Ni at 40 mMol/kg. Insects were sacrificed on day: 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16 and 20 since starting the experiment. Additionally, control animals were sampled at day 1, 12 and 16. The midgut morphology, epithelium height and the number of alive and apoptotic cells per 10800 um^2 of epithelium were examined at light microscope. The exposure to all metals significantly decreased the number of alive cells in the midgut epithelium and its height after 24 h. The number of alive cells was the lowest in Zn treatment while epithelium height was the lowest in Ni-treated crickets. Later during the experiment the epithelium showed a tendency to regeneration but in Cd-exposed crickets the epithelium height decreased later again and was completely destroyed by day 8. Degenerations of the midgut of Ni-exposed crickets was slower than in Cd-treated, and the epithelium height was the same from 4^{th} till 8^{th} day but decreased later until day 20 when the crickets died. The midgut epithelium of crickets exposed to Zn showed oscillations in height every several days and insects died by day 20. No differences between control and Zn treated animals were found in the number of apoptotic cells. It has to be noted that the number of alive cells and the epithelium height decreased also in control crickets by day 16, probably representing a natural aging effect. All metals induced degenerations of the midgut but the effects were metal-specific. Cd induced strong degenerative changes in the midgut after 1-4 days, while in crickets treated with Zn the gut epithelium was low and the proliferating layer was destroyed after 8-20 days. The exposure to Ni induced swelling of cells next to the midgut lumen and their degeneration after 1-8 days. Longer exposure induced cell swelling and degenerations also in the deeper layers of the midgut. The results confirm that metal toxicokinetics can be the direct effect of gut epithelium degeneration due to metal toxicity, as postulated by Argasinski et al. (2010). References: Argasinski K., Bednarska A., Laskowski R. (2012) The toxicokinetics cell demography model to explain metal kinetics in terrestrial invertebrates. Ecotoxicology 21: 2186-2194. Acknowledgements: Supported by the Polish Ministry of Science and Higher Education (Project No. NN304 038440) and Jagiellonian University (DS-758).

WE049

How protective is the chronic earthworm endpoint to soil arthropods in the ERA?

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According to the new commission regulation (EU) No 283/2013 (data requirements for active ingredients under the regulation (EC) No 1107/2009), the acute earthworm test is not required anymore, but the chronic earthworm test should be routinely conducted. This decision is based on results showing that reproduction of *Eisenia* (*fetida + andrei*) is more sensitive than acute mortality. Soil arthropods have to be tested if the foliar application raises concerns regarding beneficials (non-target arthropods) or the plant protection product is applied directly to the soil. The objective was to determine whether the chronic earthworm endpoint is suitable to assess the risk to soil arthropods in the first tier risk assessment for plant protection products. A literature search revealed that soil arthropods such as collembolans aremore sensitive to insecticides than *Eisenia* species. They can show less sensitivity to fungicides compared to *Eisenia* speciesandfor herbicides only a small number of studies on both organisms were available, showing inconsistent results. The findings suggest that soil arthropods should be routinely tested in the regulatory risk assessment, at least for insecticides. In this context, a survey of the Swiss ecotoxicological database (holding data on formulations and active ingredients with either approved or pending for authorization in Switzerland) was conducted. Firstly, acute (median lethal concentration, LC50) and chronic (no observed effect concentration, NOEC) endpoints of *Folsomia candida*, *Folsomia fimetaria* and *Hypoaspis aculeifer* were compared to those of *Eisenia* speciesfor insecticides, fungicides and herbicides. Secondly, regulatory accepted concentrations (RAC) for *Eisenia* species were calculated. The chronic endpoints were divided by 5, according to the environmental risk assessment of the European Union (commission regulation (EU) No 546/2011). The resulting RAC were taken for comparison to the lowest endpoints of either *Folsomia* species or *H. aculeifer*. Results are presented and further needs for first-tier risk evaluation of non-target soil organisms are discussed. \n

WE050

Physiological and molecular responses of springtails exposed to combined chemical and drought stress by passive dosing

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Organisms in the environment are exposed to multiple stressors of both natural and anthropogenic origin. Consequently, adapted organisms have developed essential physiological and molecular responses to counteract physical and chemical stress. This poster presents the results of two studies on the combined effects of phenanthrene and drought in the springtail *Folsomia candida*. Special attention was given to (1) the experimental approach for the simultaneous and independent control of chemical and drought exposure, (2) the characterization of the combined effects on survival and (3) the physiological and molecular responses to phenanthrene, drought and combined exposure. A new passive dosing system was developed and applied in order to conduct bioassays under well-defined exposure conditions: Passive dosing from silicone was used to control the chemical activity of phenanthrene (chemical stress), while saline solutions were used to control the water activity (drought stress). First, a two-factor experiment on seven levels (7^2 = 49 combinations/treatments) was conducted with the aim of studying the combined effects of phenanthrene and drought on the survival of *F. candida*. An “independent action” model was fitted to the observed survival data, which demonstrated an overall synergistic effect of the two stressors ($p< 0.0001$). Additionally, both chemical and drought stress was found to reduce the springtail tolerance to the other stressor. Then, the transcription of nine candidate genes was determined in one of the treatments from the full-factorial experiment (chemical activity of 0.010 and water activity of 0.988) with the aim of studying the effect of phenanthrene on physiological mechanisms involved in drought tolerance and, conversely, the effect of drought on phenanthrene detoxifying mechanisms. Phenanthrene had no effect on drought-protective accumulation of myo-inositol, and normal water conserving mechanisms of *F. candida* were functioning despite the near-lethal exposure level of the toxicant. Further, detoxifying induction of *cytochrome* P_{450} and *glutathione-S-transferase* was not impeded by drought. Both phenanthrene and drought induced transcription of heat shock protein (*hsp70*) and the combined effect of the two stressors on *hsp70* transcription was additive, suggesting that the cellular stress and lethality imposed by these levels of phenanthrene and drought were also additive.

Community and ecosystem ecotoxicology (P)

WE051

Effects of variable temperature and the influence of copper on Gammarus fossarum Koch, 1835

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In this study the effects of temperature increases, as well as the effects of copper ions, on *Gammarus fossarum* (Koch, 1835), a eurythermal key species of spring communities were examined. In the first experiment the water temperature was varied and the effect of these changes on *G. fossarum* observed. In a next step LC_{50}-tests with copper were conducted at different water temperatures. A sub lethal concentration of copper was chosen for the second experiment which tested the influence of elevated temperature and exposure to copper. All experiments were conducted in boxes placed in flow channels in the laboratory. The gammarids were fed with *Fagus sylvatica* leaf discs. Primarily two analytical methods were applied: the respiratory Electron Transport System (ETS) assay was conducted in order to determine metabolic changes in the test organisms; and the feeding activity of the amphipods was measured and a shredder rate calculated. The results of the first experiment show that shredder rates increased with increasing water temperature. The ETS-activity did not show significant differences at the different temperatures tested. An explanation for this finding is the ability of the organisms to adapt on a short-term basis to the changed environmental circumstances. The results of the second experiment show that the sub-lethal copper dose had no significant effects on the shredder rates, although a trend towards lower feeding activity was observed. The shredder rates increased significantly with higher water temperature. The ETS-activity was also significantly higher at the higher water temperatures, and the ETS assay demonstrated clearly that copper had a significant negative effect on the organisms. A combination of the analytical methods was found to be beneficial when testing for effects of environmental changes and pollutants on a species. The last experiment, conducted in the laboratory and field, deals with the accumulation of copper in the food source and tissue of the exposed organisms and the effects this has on their ETS-activities. This experiment is a crucial step as it is important to

conduct tests directly in the ecosystem to be able to evaluate results obtained from laboratory experiments. The implications of the results presented here for spring communities, harbouring many cold-stenothermal organisms, which are adapted to these constant habitats and sensitive to environmental changes, are then discussed.

WE052
Invertebrate avoidance behaviour as a screening tool for biochar-amended soils under viticulture
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Biochar application to soils has been proposed as a measure for increasing crop yield in drylands by reducing soil erosion and improving water and nutrient availability to plants. It is likely that Portuguese viticulture will experience increasing drought stress, as predicted by climate change models. The use of biochar in viticulture is being investigated in a field trial, as a possible strategy to help this sector in adapting to low water availability conditions. To ensure sustainable application at the large-scale, it is vital to assess any possible toxicity towards terrestrial organisms, which may lead to loss or deterioration of soil functions. With biochar ecotoxicology only now emerging, studies focusing on biochar effects on terrestrial species remain scattered and lacking in environmental, ecological and practical relevance. This study aimed at testing the suitability of avoidance behaviour assays to be used as a rapid screening and monitoring tool for evaluating the potential toxicity associated to biochar-amended viticultural soils, over a 10 month period. The selected assays employed earthworms (*Eisenia andrei*), collembolans (*Folsomia candida*) and isopods (*Porcellionides pruinosus*) exposed to different field treatments and biochar application rates for 48h, following well-established and/or standardized avoidance tests. Overall, there was no avoidance of the tested organisms to either soil treatments, with isopods and earthworms showing significant preference for biochar and biochar-compost at the field rates. Results suggest that terrestrial avoidance behaviour tests using invertebrates with different ecological functions may be adequate as an early, rapid and low-cost screening tool for toxicity assessment of biochar-amended soils, in real field applications. The assays were sensitive in discriminating between different treatments and sampling times and may complement other strategies in routine risk evaluation of biochar-enriched fields.

WE053
Prediction of salinisation effects on soil ecosystems using Terrestrial Model Ecosystem
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Soil salinisation problems are increasing with the rising of the sea level (due to the melting of glaciers and ice caps and to water expansion) and intrusion of seawater. In agricultural fields of coastal areas, both intrusion and irrigation with saltwater can threaten crop yields and the effects of these actions to soil fauna are still unknown. The aim of this study was to evaluate the effects of salinisation on soil ecosystems due to intrusion and irrigation with saltwater. To fulfill this goal, Terrestrial Model Ecosystems (TME) were used simulating two soil salinisation scenarios with a gradient of dilutions of natural seawater (3, 6, 8, 13, 18 and 30 mS/cm): 1) saltwater intrusion by immersing the lower 10 cm of the TME's in the gradient of dilutions of seawater and surface irrigation with distilled water and 2) saltwater intrusion described earlier plus surface irrigation with a gradient of dilutions of seawater. The control consisted in the immersion of the lower 10 cm of the TME's in distilled water and surface irrigation with the same liquid. Three sampling periods of soil mesofauna were established (T0, T1 and T2). The acclimation period took two weeks (after TME's extraction) during which all soil cores were under control conditions (T0). The effects of saltwater intrusion and irrigation were measured after six weeks of exposure (T1). At this point, and during eight weeks, the remaining soil cores were again submitted to control treatment to measure population's recovery (T2). Results showed some associated variability, normal of this type of studies. However, at the lowest conductivity values (highest dilutions of seawater) the abundance of soil organisms was not affected while for treatments with highest conductivity, a lower number of organisms and species richness were found when compared with the control. Despite the resistance of the soil species to low salinisation levels, some soil functions can be affected at field scale in a real scenario due to the ability of some species to avoid highly saline places.

WE054
Intraspecific variability and biodiversity sustenance along toxicity gradients
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The conservation of biodiversity is frequently included among the protection goals of environmental legislation. Chemicals may affect biodiversity but their potential effects on biodiversity are at present poorly understood, as the mechanisms relating toxicity to diversity have not been adequately explored. Here, we present a new community model integrating demography, dispersal and toxicant-induced effects on reproduction driven by intraspecific and interspecific variability in toxicity tolerance. We compare model predictions to 458 species abundance distributions (SADs) observed along concentration gradients of toxicants to show that our model is able to capture toxicity-induced biodiversity changes of herbicides and metals in algal communities. We also show that the best predictions occur when intraspecific variability is set five to ten times higher than interspecific variability. At high concentrations, lower settings of intraspecific variability resulted in predictions of community extinction that were not supported by the data. Our results propose intraspecific variability as a key driver for biodiversity sustenance in ecosystems challenged by environmental change.

WE055
Ecological interactions alter the sensitivity of Daphnia magna populations to chemical stress.
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The objective of ecological risk assessment (ERA) is to quantify the risk that a given chemical exposure would affect ecosystem functioning and structure. However, the currently used lower-tier effect assessment approaches are insufficient because they are ecologically unrealistic and/or not cost-efficient. To better understand how ecological interactions affect the response of communities to chemicals, we exposed microcosms containing (i) different starting population densities of a grazer, (ii) two competing grazers and (iii) a grazer and its predator to pyrene, and monitored the population dynamics over several weeks. The experiments were conducted using *Daphnia magna* and the rotifer *Brachionus calyciflorus* as grazers and *Chaoborus* larvae as a predator on *D. magna*. Experiments were conducted with one week of pre-treatment, two weeks of exposure period, and two weeks of recovery. *D. magna* was the most sensitive species ($EC_{50,immobilization}$ = 68 μg/L), while *B. calyciflorus* and *Chaoborus* larvae showed no effect on survival at pyrene concentrations up to 200 μg/L. Pyrene was added to the microcosms at three concentration levels: 10, 25 and 75 μg/l in week 2 and 20, 50 and 150 μg/L in week 3. Generalized linear models (GLMs) were used to assess the effects of pyrene and the species interactions at different time points. Four days after the first pyrene addition, pyrene had a significant but limited effect on *D. magna* abundance (< 6% variance explained). The second addition of pyrene resulted in a larger effect where pyrene explained up to 72% of the variation. Even when exposed to pyrene, the population dynamics of *D. magna* were mainly determined by predation. The competition with *B. calyciflorus* became insignificant after three weeks, not surprising given that *B. calyciflorus* were outcompeted. Intraspecific competition had a negative effect on *D. magna* densities after 14 days, independent of the presence of pyrene. Increased competition for the same resource thus negatively affected population densities. As expected, intraspecific and interspecific competition and predation altered how *D. magna* populations respond to pyrene. Surprisingly, the ecological interactions tested here (competition and predation) decreased the percentage of D.magna affected by pyrene. This study offers empirical evidence that ecological interactions can play a significant role in how populations respond to chemical stress.

WE056
Tolerance on a plate – a novel assay using natural bacterial communities to estimate increased long-term tolerance to multiple chemical stressors using MT2 microplates
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Bacterial communities play an important role in aquatic ecosystems and also provide convenient small model ecosystems in order explore the ecological consequences of toxicant exposure. Microbial communities can be used to study effects of toxicants under more realistic conditions than single species tests and overcome some of their limitations as a multitude of taxa are included and ecological interactions are allowed to affect the test results. The PICT concept postulates that a community with a history of chemical exposure shows an increased pollution tolerance, but only to the historically present compounds or closely related chemicals with similar ecological modes of action and/or tolerance mechanisms. The general idea of the presented tolerance assay is to sample natural bacterial communities from a pristine site and from a site with a history of pollution events, and to then compare their tolerance fingerprints by exposing them to a suite of toxicants with different modes of action. In order to overcome a major limitation of classical PICT studies, the reliance on short-term tests for querying the tolerance development, we explored the use of MT2 plates that allow to perform 96 carbon source utilization tests in a microtiterplate: each well contains tetrazolium violet (a redox dye that colormetrically indicates respiration), growth medium and a certain concentration of a specific "challenge toxicant". The color development in each well after an incubation of up to 48hrs then gives an integrated measure of how much growth and respiration of the bacteria is affected by the particular challenge toxicant present. An increased color development of a community from a polluted sited hence indicates tolerance to a certain class of challenge toxicants. The resulting multivariate tolerance pattern consequently allows to establish a causal link to the classes of pollutants that are present at a site in ecotoxicologically relevant concentrations. As a first step towards assessing the proposed method we employed biofilm and planktonic communities that were pre-exposed in microcosms for several weeks to either the antibiotic ciprofloxacin, ionic silver or silver nanoparticles. "Challenge toxicants" comprised silver, the antibiotics ciprofloxacin, ofloxacin, and sulfamethoxazole as well as the pesticide paraquat. Finally, NaCl was included as an unspecific toxicant causing osmotic stress.

WE057
Trophic niche metrics of gastropods (Lymnaea stagnalis) in pond mesocosms exposed to hydrocarbons: A new sensitive endpoint for dose-response characterization?
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Trait-based approach in Ecological Risk Assessment (ERA) has been recently promoted. Trait distribution and variations are recognized as potential integrators of environmental changes. They may be divided into biological traits linked to species intrinsic characteristics (*i.e.*, respiration, life cycle) andecological traits, in relation with biotic, abiotic and functional processes (*i.e.*, amplitude of trophic niche, litter breakdown). Descriptors of trophic niche and of food web properties have been suggested as potential integrative and sensitive endpoints of toxicant effects. Among the various methods available for describing trophic niche, a large amount of work in ecology has promoted those based upon stable isotopes as a proxy of the realized trophic niche, including through quantitative metrics. In the present study, carbon and nitrogen stable isotope signatures were used to assess the effects of a Hydrocarbon Water-Accommodated Fraction (HWAF; 0.01, 0.4, 2 and 20 mg/L nominal loadings) on the realized trophic niche of freshwater gastropods (*Lymnaea stagnalis*) in pond mesocosms. Snails were sampled before the beginning of treatment, immediately at the end of the treatment period (one pulse per week for 4 weeks) and 5 months after the end of the treatment period. Biofilm stable isotope signature was used to provide a baseline to standardize gastropod signature. Various trophic niche metrics were calculated and showed a high sensitivity to HWAF, but exposure resulted in different response patterns according to the sampling time and loadings. HWAF clearly affected gastropod trophic niche leading to change in the food resources used and resulting in trophic niche bursting (*i.e.*, increase of diversity of used resources) or trophic niche collapse (*i.e.*, decrease of diversity of used resources) across time and loadings. The sensitivity of each trophic niche metric was investigated after the treatment period and at the end of the experiment. It highlighted a clear dose-response relation following a hormesis-like pattern. These results suggest that trophic niche metrics based on stable isotopes offer a promising way to investigate sublethal effects of hydrocarbons and subtle disturbances in food web and community functioning. Relevance of these new endpoints in ERA is discussed.

WE058
A model ecosystem experiment to study the recovery capability of a macrozoobenthos community after a pesticide stress
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The recovery capability is a fundamental component of the vulnerability of populations and communities, particularly relevant in case of non-continuous stress factors, such as pesticide pollution. The present work was performed using an experimental system composed of 5 artificial streams (flumes), fed by unpolluted river water, which reproduced a mountain river ecosystem under controlled conditions. The environmental conditions are comparable to those of rivers in the same geographic area, subject to pesticide pollution from intensive agricultural basins, mainly apple orchards and vineyards. Three flumes were exposed for 24 hours to chlorpyrifos (nominal concentration: 1.5 mg/L), other two flumes were the untreated controls. After exposure, at the inlet of two of the treated streams and of one control stream, a metal-net filter system was posed in order to avoid recolonisation by drift. In the other two flumes drift was allowed. So, the specific role of drift and direct colonisation in the recovery capability may be assessed. To avoid obstruction, the filter systems were cleaned daily. The experiment started early July 2013 and the community was sampled for five weeks after stopping exposure. The community structure was checked by periodical sampling using a Hess sampler. Drift at the outlet of the flumes was also sampled regularly. Samples were taken in all flumes before exposure, immediately after exposure stop, 24 hours after exposure and then every 4 days up to the end of the experiment. Selected taxa were also sampled, for the measurement of a set of biochemical parameters (e. g. acethilcholinesterase, glutathione-S-transferase, alkaline phosphatase, and catalase) before exposure, immediately after and 24 hours after exposure. During exposure, water samples were taken for the analytical control of concentrations. The exposure to the pesticide caused a significant increase in the drift in most of the taxa present in the flumes. This produced a dramatic decrease in abundance and diversity of the benthic community at the end of the exposure period. Three weeks after exposure, the new colonisation was almost complete, and the benthic community was restored in all the flumes. Preliminary results suggest a low resistance but a good resilience of the macro-zoobenthos community.

WE059
Environmental stressors can enhance the development of community tolerance to a toxicant
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Ecosystems are subject to a combination of recurring anthropogenic and natural disturbances, such as climate change and pesticide contamination. Biological communities are known to develop tolerance to recurring disturbances due to successive changes at both the community and organismal levels. However, information on how additional stressors may affect the development of such community tolerance is scarce to date. We studied the influence of hydrological disturbance on the reaction of zooplankton communities to repeated insecticide pulses in outdoor pond microcosms. The communities were exposed to three pulses of the insecticide esfenvalerate (0.03, 0.3, and 3 μg/L) and to the gradual removal of water and its subsequent replacement over three cycles. The communities developed tolerance to the toxicant, as indicated by their decreasing reaction to subsequent contamination, and this development was enhanced by hydrological disturbance. Elimination of the key taxa Daphnia spp. through the combined action of the two stressors was identified as the main mechanism responsible for the increase in community tolerance under a fluctuating water level. Under a constant water level, the abundance of Daphnia spp. did not consistently decrease following the contaminations, indicating that other mechanisms were responsible for the observed community tolerance. The present study shows, for the first time, that additional stressors can facilitate the development of community tolerance and that such facilitation is propagated through community-level mechanisms. For details see: Stampfli N.C., Knillmann S., Noskov Yu.A., Schäfer R.B., Liess M., Beketov M.A., 2013. Environmental stressors may enhance development of community tolerance to a toxicant. Ecotoxicology, in revision.

WE060
Towards a better understanding of algal ecology in ditches and streams -Monitoring phytoplankton and periphyton in agricultural landscapes-
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Algae as primary producers, are of crucial importance especially in small lotic systems, where autochthonous material is the main carbon source. Considering their importance, very little is known concerning their population dynamics in small streams and ditches within seasons. This is especially true for phytoplankton populations, since they are thought to be of minor importance in small lotic systems

and are therefore sparsely implemented in monitoring programs. This study targets the gap of knowledge and aims to provide further information on phytoplankton and periphyton population dynamics and seasonality, in order to support monitoring actions and decision making within regulatory processes. The study was carried out in the Vogelsberg region in central Germany in an agricultural landscape. Phytoplankton and periphyton samples were taken from five streams at ten sampling sites in bi-weekly intervals over a one or two year period. Algal analysis was performed with delayed fluorescence spectroscopy, which is a real time non-destructive method to determine total chlorophyll concentrations and distinguish four colour classes of algae. Principle response curves were used as a statistical method for detecting seasonal differences in algal populations by fixing one sampling date as an internal reference. A clear seasonal pattern of phytoplankton and periphyton communities was revealed at all sampling sites. This seasonal pattern was consistent when comparing the two years as well as when comparing the different sampling sites and streams. To compare algae communities in time between sites second principle component analyses were performed, using one sampling site as the reference. It could be shown that in-stream variability (variability between sites of one stream) is similar to between stream variability. The algal distribution and community structure at the sampling sites behave similarly throughout the year, which implies that seasonality in small lotic systems in agricultural landscapes in temperate regions can be considered as a recurring, generalizable phenomenon. Additionally, it could be shown that algae populations start growing very early within a year (March) and that after a disturbance (dry out period) the recovery to the expected state is rapid. The information concerning algae population dynamics and the insights into primary production, highlight the value of algae monitoring throughout the year in field studies.

WE061

MicroCokit: Microbial Community-based indicator of water quality to integrate in a modeling scenario

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Microbial communities are the base of the food web pyramid, representing about 50% of the total biomass on Earth. They are responsible for the geochemical cycles and bio-removal of organic compounds and xenobiotics playing a key-role in the ecosystem function. Their capability to adapt quickly not only to the changes but also to take the advantages, makes them the drivers of the aquatic and terrestrial ecosystem and the human health. In Europe, the good quality of surface waters is established under the Water Framework Directive (WFD) based either on the chemical monitoring, or on the ecological status and pathogen detection in drinking and bathing waters. However, no indicator is foreseen which would provide a link between ecological and chemical pollutants and other anthropogenic pressures; currently water quality monitoring are either focused on ecological parameters or only chemical pollutants. The MicroCokit Project, a Marie Curie Industry-Academia Partnerships and Pathways (FP7-PEOPLE-2012-IAPP), a close collaboration of Academic groups with European Commission Joint Research Centre and leading private Enterprises, has been conceived to i) investigate and identify complex stressor indicators based on microbial communities; ii) foster the transfer of knowledge among the partners with the final goal to bring to market faster, more sensitive and robust tools as bioindicators of water quality. The tools will be developed according different kinds of bioindicators, targeting microbial community, pathogen and specific microorganisms. For this purpose the river Tiber has been chosen as a pilot case study and sampling sites were selected based on different anthropogenic pressures which they are exposed to. For each site water sample will be analyzed for both microbiological (Microarray, Metagenomic and FISH analysis) and chemical analysis (organic and inorganic compounds, including emerging pollutants). Following the validation, these data could be then integrated in a modeling system to predict, prevent and mitigate the impact of anthropogenic pressure on water management.

WE062

Sensitivity of Functional Diversity Indices (FDI) in higher-tier ERA: Cross comparison of macroinvertebrate traits in stream and pond mesocosms exposed to a fungicide and a petroleum distillate

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Complex responses may be observed at the community level following exposure to chemicals due to a combination of direct and indirect effects affecting functional processes. The nature and intensity of these effects depend on various factors including the characteristics of the ecosystem and the traits of exposed taxa. Chemical exposure may change environmental conditions leading to a shift of "environmental trait filter" with echo on biological and ecological trait distribution of the species present in the systems. To address such effects, Ecological Risk Assessment (ERA) has to include endpoints that are more closely linked to ecosystem functioning. n this study, various trait-based indices were used to characterize biological and ecological traits distribution in benthic macroinvertebrate communities exposed to chemical in outdoor stream and pond mesocosms. Trait analyses were performed on potentially impacted traits with respect to the modes of action of the tested chemicals. Thiram, a dithiocarbamate fungicide, and the hydrocarbon fraction of a petroleum distillate were injected into ponds and streams at the same range of exposure concentrations (35 and 170 μg/L for thiram and 0.01, 0.4, 2 and 20 mg/L for hydrocarbons) for both types of systems. All treatments were performed in duplicates and four systems were kept as the controls. Streams were continuously treated for 3 weeks. Ponds were treated weekly for 4 weeks. The exposure period was followed by a 2- or 10-month recovery phase for streams and ponds, respectively. Macroinvertebrates were sampled weekly or every 3 weeks in streams and ponds, respectively using artificial substrates. Multidimensional functional indices, including functional richness (FRic), functional divergence (FDiv), and functional evenness (FEve), were calculated. Functional diversity was estimated using Rao's quadratic entropy (RaoQ) and functional dispersion (FDis) was also computed. Most of these FDI showed a high variability explained by both time and treatment. Four types of FDI response patterns were observed: specific case sensitivity (one chemical, one type of mesocosm), specific aquatic system sensitivity (both chemicals, one type of mesocosm), specific chemical sensitivity (one chemical, both types of mesocosms) and general sensitivity (both chemicals and types of mesocosms). Nevertheless, FDI did not show a strong discriminating power between the seasonal and exposure effects.

WE063

The ecological consequences of various chemical emission patterns in food webs

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The objective of ecological risk assessment is to quantify the risk a chemical may pose to ecosystem structure and functioning. Current approaches are insufficient because they do not consider dynamic exposure patterns, ecological interactions e.g. competition and predation, and the indirect effects resulting from these interactions. Given the large quantity of chemicals that need to be assessed, fast and cheap methodologies are needed that can accurately predict the effects of chemicals on communities. Predictive modelling categorizes as such a methodology. In this study we explore ecological effects of various emission profiles in a simple freshwater food web by coupling a basic chemical fate model with a food web model. The chemical fate model is based on the "Quantitative Water Air Sediment Interaction" or "QWASI" model for a lake. The QWASI-model is a fugacity based model that describes the distribution of an organic chemical over the air, water and sediment compartment. Here, the unsteady-state version of the model was used. The required model parameters are dependent on the chemical (e.g. Henry constant) and the scenario used (e.g. water volume, amount of emission). The functional groups in the food web are described using ordinary differential equations. The food web we focus on is based on a lake and includes the functional groups phytoplankton, grazers, detrivores, omnivores and predators. Differential equations are linked to each other based on the trophic interactions in the food web using functional response relationships. The toxic effect of a chemical on the different functional groups is described using dose response relationships. Using the input from different scenarios, we evaluated how chemical discharges will drive exposure concentrations in water and their effects are on the food web. Because of the food web perspective we adopted, we were able to assess both direct and indirect effects of a given chemical. The results provided here show that a food web based approach has great potential for use ecological risk assessment of chemicals.

Acknowledgement - The Chimera project is financed by the Long Range Initiative of CEFIC (www.cefic-lri.org) (project code: LRI-ECO19)

WE064

Simulation of the environmental situation of the tributary ecosystem Daninghe River in the Yangtze Three Gorges Area

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After the impounding of the Three Gorges Reservoir (TGR), located in the Chongqing and Hubei provinces in Central China, the huge impact of the dam on geo-hydrological conditions in this landscape, which is in large parts used for agriculture, is obvious. The aim of the presented research project is a deeper understanding of the processes that determine the bioaccumulation and biomagnification of organic pollutants, i.e. mainly pesticides, in aquatic food webs under the newly developing conditions of the TGR. In our modeling studies, we describe the impacts of nutrients and pollutants by comparing environmental scenarios adapted to the area of the Daninghe River as a typical Yangtze tributary. For this purpose, we calibrated and adapted the US-EPA model AQUATOX to the specific situation in our model region. In order to test combinations of environmental factors (nutrients and pollution) and to study the potential accumulation and biomagnification, we used an integrated modelling approach, which contained several specialized modules addressing the complexity of the TGR-situation concerning hydrological, biological and ecotoxicological conditions. The patterns that emerge from the model calibration to Yangtze conditions are stable over a period of more than 30 years. It was investigated to what extent the nutrient loads have to be reduced to minimize the risk of frequent algal blooms that affect the water quality severely. Indicators for environmental and consumer risks, resulting from the model pollutants, will be deduced from the modeling results. From this, proposals of risk management measures as well recommendations for the melioration of the TGR's water quality and for the reduction of pollution loads in sensitive areas are developed, e.g. the prevention of pesticide overuse and the establishment of waste water treatment plants. Emphasis is given on recommendations for consumers, e.g. to give advice how to avoid exposure by high pollutant burdens of food, esp. fish. The potential bioaccumulation of important food sources (e.g. the 'Chinese carps') will be compared to acceptable daily intake for chronic exposure as deduced from an environmental risk assessment of a model herbicide (Propanil) and its metabolites under different pollution scenarios.

WE065

Consecutive dry-wet cycles influence toxicity of aqueous fractions of biochar-amended soil

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There is increasing interest worldwide in biochar application to soils, in order to enhance productivity and other soil functions, as well as carbon sequestration. While forecasted global changes predict more frequent cycles of soil drying-wetting, the result may be an enhanced risk to aquatic ecosystems over time, from exposure to run-off and leachates from biochar-enriched soils. This study aimed at: i) developing a suitable methodology based on consecutive soil dry-wet cycles and water-extraction, to simulate enhanced bioavailability of metals and PAHs in aqueous biochar-amended soil fractions; and ii)evaluating acute toxicity of such fractions towards representative aquatic organisms, using a battery of established and/or standardized bioassays (e.g. bioluminescence inhibition of the marine bacteria *Vibrio fischeri* - Microtox®; growth inhibition of the microalgae *Pseudokirchneriella subcapitata* - OECD, 2006; and immobilization of the invertebrate *Daphnia magna* - OECD, 2004). Total metal and PAH concentrations in soil-biochar aqueous extracts generally met current EU regulatory benchmarks for surface waters. Nonetheless, there were effects on the tested organisms (based on estimated effect concentrations - EC_{50}, EC_{20}, EC_{10}), with dose-response patterns depending on species and number of dry-wet events. Acute toxicity from exposure to aqueous soil-biochar extracts depended on the number of cycles for all tested organisms. Overall, results suggest that on the short-term, frequent dry-wet cycles may lead to increased risk to aquatic ecosystems exposed to aqueous fractions of biochar-enriched soils. A battery of aquatic assays that are simple, rapid and inexpensive was suitable to complement traditional chemical characterization of soil-biochar aqueous extracts therefore, increasing the ecological relevance in risk assessment of biochar-amended soil. To the best of knowledge, this is the first study to describe an ecologically relevant approach for addressing the potential risks to aquatic ecosystems exposed to run-off and leachates from biochar-enriched soils, subjected to consecutive drying-wetting events.

WE066

Effect of different environmental regimes on the fate and isomeric fractionation of the flame retardant HBCDD in experimental aquatic ecosystems

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Hexabromocyclododecane (HBCDD) is an additive brominated flame retardant used mainly in polystyrene foam, textiles and electronic appliances. It is considered a persistant, bioaccumulative and toxic substance. In the aquatic environment, HBCDD has been found to partition mainly to particulate matter and sediment and also to associate with lipids and biomagnify in aquatic food webs. However, the influence of environmental factors on partioning in field or semi-field conditions is poorly understood. We studied the partitioning of HBCDD added in a particulate suspension to experimental aquatic (coastal) ecosystems in three different experiments. Effects of elevated temperature, season and presence or absence of macrofauna in the sediment were examined. Using GC-MS and LC-MS, we measured the concentrations and isomeric fractionation of HBCDD in water, suspended particulate matter, sediment and biota in the ecosystems. The experimental ecosystems were assembled from shallow coastal bays in the Baltic Proper and comprised sediment (with infauna), overlying water (including plankton and particulate matter) and in one experiment also macrophytes and macroalgae. In all three experiments, HBCDD partitioned mainly to the sediment, and this proportion increased with time. In the water, more than half of the HBCDD was found in the particulate phase, even 8 months after the addition, but total concentrations in this phase decreased with time. The presence of macrofauna in the sediment increased the concentration of HBCDD in the sediment and decreased its concentration in the water. Filter- and deposit feeding infaunal bivalves (*Macoma balthica*) could contain up to c. 10% of the total amount of HBCDD added. Increased temperature (5°C above ambient for 2 weeks) decreased the amount of HBCDD in the sediment and in the water but not in *M. balthica*. The sediment was generally enriched in the beta isomer, while the dissolved phase of the water and *M.balthica* were generally enriched in the gamma isomer. Much of our knowledge of partitioning and fractionation of HBCDD comes from field studies. By using carefully controlled experiments containing natural coastal assemblages and a realistic exposure scenario we have been able to determine more accurately the fate of this substance in the ecosystem, as well as the influence of benthic-pelagic coupling, temperature and time/season.

Biodegradation and Environmental Fate of Chemicals - Regulatory Acceptance of Non-Standard Tests (P)

WE067

Assessing the Persistence of Chemicals in the Environment: Future Research Priorities

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Many chemical regulatory schemes exist around the world that contain hazard-based criteria to identify and prioritise persistent, bioaccumulative and toxic (PBT), or very persistent very bioaccumulative (vPvB) chemicals. A two-day workshop, co-sponsored by ECETOC and the CEFIC Long-range Research Initiative (LRi), was held to discuss the state of the science and future research needs associated with assessing the environmental persistence of chemicals. The discussions held at this workshop clearly indicated that the science- and knowledge-base within the field of persistence assessment was moving forward. Specific examples where significant development has taken place included, the ECETOC and UBA activities to define and characterise extractable and non-extractable residues (NERs) formed in soil and sediment, the CEFIC funded work to understand the importance of biomass diversity within screening assessments for biodegradability, and the inclusion of more ecological realism within persistency assessments through the inclusion of light, natural waters and adaptation potential. This poster will highlight the outputs from the syndicate sessions within this workshop. These sessions focused on the future challenges and prioritised future research and development requirements including: (i) convening an OECD Expert Working Group to consolidate and update the RBTs to reflect the availability of new instrumentation with increased analytical sensitivity; (ii) assessing the impact that temperature has on the rates of biodegradability in aquatic and aquatic-sediment habitats to determine the scientific basis for temperature extrapolation; (iii) demonstrating the ecological significance of adaptation and developing appropriate test guidelines and guidance for its inclusion within persistence assessments; (iv) developing and validating models to predict non-extractable residue formation and guidance on how to assess the risks posed by NERs over time; (v) evaluating the microbiological, kinetic, ecological relevance and performance of the OECD 314, 308, 309 and enhanced biomass tests using appropriate reference and benchmark chemicals; (vi) developing and validating tools and guidance to predict the formation of transformation products in biodegradation studies; (vii) investigating the value of metagenomics to determine the relevance of laboratory inocula in order to interpret and understand test outcomes to improve and refine the test systems.

WE068

Refinement of Biodegradation Tests to Prepare for Subsequent E-Fate Testing and Assessment

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Many years of ready biodegradation test conduction and the use of such studies for several finalities of the test items have revealed a number of possible options to learn as much as possible from the tests according to OECD TG 301 A-F. First it is recommendable to gather information on possible microbial toxicity of the test item, e.g. from literature, conclusion from mode of action or the results from other tests (e.g. OECD TG 209, 216, 217, 224 and/or ISO 10712). Equilibrium Partition Method may be used to conclude on pore water concentrations from terrestrial tests. Minimisation of the test item concentration may be considered as a possible solution. Particularly when the degradation curve shows a plateau formation without reaching the pass level, it may be worth to survey in addition the DOC in tests monitoring respiratory parameters and to apply analytical investigations to follow the parent substance. Such analytics may be used to confirm bioavailability of the test item and non-mineralised transformation products. Literature data and in silico tools can deliver information on possible degradation steps and to search analytically for the resulting chemicals. Sterile controls may serve to evidence absence of abiotic transformation or to justify a prolonged lag-phase in case there is e.g. primary abiotic degradation interrupting the bioavailability of the parent test item. Some of the test designs allow prolongation of the test duration, which can indicate potential for inherent and/or ultimate biodegradation or serve to detect a stable transformation product. Additional controls can be used to show that a compound is readily degraded as a nitrogen source only, e.g. by a procedure control without inorganic nitrogen.

WE069

Novel simulation test to assess the primary and ultimate biodegradability of chemicals in sediments: Application to surfactants

K. McDonough, PG / Environmental Stewardship; N.R. Itrich, The Procter Gamble Company / Environmental Stewardship Organization; E. Schwab, The Procter Gamble Company; T.W. Federle, The Procter Gamble Company / Environmental Stewardship

The development of specific regulatory persistence criteria and a growing need to conduct risk assessments in sediment has increased the need to better understand fate in this compartment. A novel simulation method has been developed to assess the fate of chemicals in aerobic and anaerobic sediments under realistic conditions. Small replicate microcosms containing 1 mL of sediments with 0.1 mL of overlying water were dosed with tracer concentrations of radiolabeled tetradecanol, alkyl sulfate (AS), alkyl ethoxylate (AE), alkyl ethoxylate sulfate (AES), and linear alkylbenzene sulfonate (LAS). For each chemical being evaluated, the microcosms were incubated together in a flow-through system to trap evolved $^{14}CO_2$. Periodically, individual microcosms were analyzed for dissolved $^{14}CO_2$, parent, metabolites and bound residues. In all cases, mineralization was closely linked to parent disappearance with little accumulation of metabolites. This method provides the kinetic data needed to evaluate primary and ultimate biodegradation in undisturbed sediments as well as information on biodegradation intermediates and end-products. Unlike tests using sediment-water slurries or sediment microcosms with a large volume of overlying water (OECD 308), this test allows the economical and time efficient assessment of primary and ultimate biodegradation in an undisturbed sediment phase. The smaller test vessels, reduced glassware, and whole system trapping apparatus make it feasible to collect a larger number of sample points which is critical in obtaining accurate primary and ultimate biodegradation kinetic data.

WE070

The AnBUSDiC Test; a New Screening Method for the Anaerobic Degradability of Surfactants

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Surfactants are widely used across the globe both in industrial and consumer products, their biodegradation characteristics are therefore of high importance. Upon entering a WWTP the majority of surfactants are aerobically mineralized to CO_2 and H_2O. However, a small fraction is inevitably left non-degraded and adheres to the remaining sludge. This sludge is usually further treated in anaerobic digester tanks. Assessment of existing methods for determining anaerobic biodegradability has led to the development of a new test method, which is in principle based on the method DIN 38414 part 8. This new test, named the Anaerobic Biodegradation Under Sludge Digester Conditions test (abbreviated to AnBUSDiC test) allows for a quantification of the degradation of surfactants under conditions encountered in the anaerobic digester tank of municipal WWTPs. The AnBUSDiC test has several advantages over existing methods. The main advantage is that it is particularly suitable for surfactants, because the two-step design minimizes possible unspecific digester gas formation caused by the surface-activity of the test substances, therefore avoiding false positive results. In order to further standardise the AnBUSDiC test and gain regulatory acceptance, a ring test was organized involving seven laboratories, and five model surfactants from different surfactant classes (anionic, non-ionic (branched and linear) and amphoteric) plus a positive control, glucose. The AnBUSDiC test produced reliable repeatable results between laboratories however some additional modifications were suggested. It was identified that the original test method did not identify a clear endpoint from which a biodegradation value should be taken. It was proposed that a new more concise endpoint be defined in combination with the AnBUSDiC test to allow better comparability between test results. The inclusion of a second addition of test substance is a major step forward in the elimination of the variability produced by non-specific gas production. With the exception of one anomalous result for LAS, for which an explanation could be provided, the AnBUSDiC method appears to provide overall robust and interpretable results.

WE071

An assessment of biodegradability of quaternary carbon containing fragrance compounds: Comparison of experimental OECD screening test results and in silico prediction data

M. Seyfried, Biotechnology Department; A. Boschung, Firmenich SA / Biotechnology Department

The biodegradability of quaternary carbon containing fragrance substances was assessed using the OECD 301F screening test and prediction models (Biowin 1-6 individually and in combination as well as Catalogic 301F and 301C models). Despite an expected challenging profile, 37% of the test compounds met pass level after 28 days in the OECD 301F test, while another 26% indicated partial breakdown (as indicated by ≥20% biodegradation in the test). Structures could be primarily grouped into four distinct families: Cyclic and acyclic *tert*-butyl containing structures, monoterpene-resembling structures with bridged cycles, sesquiterpene-resembling structures and cyclohexane/-hexene/-hexadiene structures containing a geminal dimethyl. For a number of compounds for which structural analogs were available, it was found that structures which were rendered less water soluble by either the presence of an acetate ester or the absence of oxygen tended to be degraded to a lesser extent compared to the primary alcohols or oxygenated counterparts under the test conditions applied. Difficulties were encountered when attempting to correlate experimental with *in silico* data. While Biowin model combinations currently recommended by regulatory agencies did not allow for a reliable discrimination between readily and non-biodegradable compounds, only a comparably small proportion of the chemicals under study (30% and 63% depending on the model) fell within the applicability domain of Catalogic, a factor that critically reduced its predictive power. According to these results, currently neither Biowin nor Catalogic accurately reflects the potential for biodegradation of fragrance compounds containing quaternary carbons.

WE072

Improving biodegradation of low solubility chemicals: What can we do?

C. Mead, Harlan Laboratories Ltd / Ecotoxicology dept

Many of the biodegradation tests used for the routine assessment of the biodegradability and persistence of organic chemicals in the environment were developed a number of years ago using 'ideal' chemicals e.g. readily water soluble, non-volatile. The OECD 301 A – F series of tests are one such example of these routine tests that have become mainstays in many regulatory schemes and the results of these tests may be used for hazard, risk and persistency assessment. Due to the widespread acceptance of test data from such studies it has become more important over the years to show that biodegradation occurs in these standard tests, however it has long been accepted that limited bioavailablity of poorly water soluble organic compounds can lead to lower than expected biodegradability. Guidance documents such as ISO 10634 and REACh R7b along with published research give examples of a number of techniques that can be utilised to increase the bioavailability of such compounds. In this presentation a summary of several of the techniques employed in regulatory biodegradation tests and the influence that they can have on the biodegradation of low solubility chemicals is given.

WE073

Comparison of OECD 314B die-away results with OECD 303A simulation study results and field data

K. McDonough, PG / Environmental Stewardship; N.R. Itrich, The Procter Gamble Company / Environmental Stewardship Organization; T.W. Federle, The Procter Gamble Company / Environmental Stewardship

The OECD 314B Activated Sludge Die Away Simulation Test can be used to generate rates of primary and ultimate biodegradation of chemicals during secondary activated sludge wastewater treatment. These rates can be combined with sludge sorption coefficients and used as inputs into a wastewater treatment plant (WWTP) model such as SimpleTreat to estimate removal in an activated sludge WWTP under any set of operating conditions. The OECD 314B can also be used to determine the maximum extent to which a chemical can degrade in activated sludge WWTP with sufficient residence time. Estimates of removal in an activated sludge

WWTP can also be obtained in the laboratory from an OECD 303A Aerobic Sewage Treatment Simulation Test and through field monitoring. The focus of this analysis is to evaluate the ability of the 314B degradation rates used in conjunction with a WWTP model to accurately predict WWTP removal when compared to data for the same chemical from an OECD 303A Simulation Test and field monitoring studies. For example, predicted removal of parent linear alkylbenzene sulfonate (LAS) in an OECD 314B ranged from 99.4 to 99.6% while measured removal in an OECD 303A ranged from 99.3 to 99.4%. Field data from North America and Europe from 58 activated sludge WWTPs showed a range of removals from 97.9 to 99.9% with an average removal of 99.3%. Besides LAS, this analysis will encompass a variety of personal care product industry important chemicals with a range of physical and chemical properties and biodegradation rates such as polycylic musks and anionic surfactants.

WE074

Biodegradation of selected compounds in different water-sediment systems – water-sediment screening tool (WSST) vs. simulation studies

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The environmental fate of a compound is determined by transformation and distribution processes which are strongly dependent on the specific environmental conditions. Information on the biodegradability of chemicals in different environmental compartments is one of the major determinants of their environmental fate and therefore plays a crucial role in regulatory decision-making. Simulation studies according to OECD 308 (aquatic sediment systems) and OECD 309 (surface water) are an integral part of tiered testing strategies in different legislative frameworks. However, several shortcomings of the OECD 308 have been identified and discussed over the years that hamper the interpretation and use of the results (e.g. a strong redox gradient in the sediment and an unrealistically high sediment:water ratio). The OECD 309 suspended sediment test is strongly related to OECD 308, but might exhibit several advantages over OECD 308. However, there are only limited experiences available with OECD 309 so far. Against this background, the aim of the Cefic-funded project LRI-ECO18 is to understand the value and information content of the existing OECD 308 and 309 protocols and to develop an improved testing strategy to obtain robust degradation data for assessing persistence in sediment and water. In combination with a data analysis approach, an experimental approach is used to enable disentangling sorption from (bio)degradation and clearly distinguish between aerobic and anaerobic conditions. Therefore, a suite of five complex to less complex water-sediment systems is used to investigate the behavior of chemicals with varying degrees of sorption and biodegradability: (1) OECD 308 standard protocol (water:sed ratio = 3:1, not stirred); (2) OECD 308 modified protocol (water:sed ratio = 10:1, stirred water phase); (3) OECD 309 modified protocol (water:sed ratio = 100:1, stirred); (4) OECD 309 standard protocol (water:sed ratio = 1000:1, stirred); (5) Water-Sediment Screening Tool (WSST). This poster focuses on the WSST, which was developed based on the respirometric ready biodegradability test according to OECD 301C by integrating an artificial sediment compartment. The test system is characterized by a simple test design, a test duration of 28 days and comparatively low costs. The poster describes the test design of the WSST and presents results for selected compounds in comparison with results from the OECD 308 and 309 simulation studies.

Environmental biodegradation rates and pathways: Dependence on environmental conditions (P)

WE075

New Approach for Active Biomass Measurement and Dynamics of Bacterial Communities in Sediment

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The degradability of chemicals in different environmental compartments is one of the major determinants of their environmental fate and therefore plays a crucial role in regulatory decision making. In the study of chemicals degradability, simulation tests present several uncertainties with regard to performance, evaluation and data interpretation. In this context, this work aimed to evaluate an alternative method, based on the 16 S RNA, to measure sediments microbial activity, responsible of biodegradation through sediment-associated microorganisms.Two natural sediments, with different textures, were selected to simulate the biodegradation of four different test substances (Aniline, Pyriproxyfen, Voriconazol, Celecoxib). For every sediment/test substance, four tests were conducted in parallel including: a setting according OECD 308, OECD 308 modified (with a thinner, ideally fully aerobic sediment layer), OECD 309 and OECD 309 modified (with higher sediment content). Biomass was measured, at the beginning of and at the end of test by fumigation and also using reverse transcriptase quantitative PCR (qRT-PCR). Additionally the microbial diversity of the sediments was studied with polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Eubacterial primers were used, in both, qRT-PCR and DGGE (GC clamped primers). In order to monitor the function of the microorganism in each test system the mineralization of ^{14}C-Aniline as reference standard was measured. This way a huge data set will be finally available to compare the different methods for microbial activity determination. The results obtained so far showed no correlation between the mineralization, fumigation and qRT-PCR results, and in most cases biomass was overestimated when using the fumigation method. The results of the qRT-PCR did not show significant differences between the different test settings, however different microbial profiling was observed in the DGGE pattern. This analysis of microbial diversity gives first indications of a possible shift of individual microorganism populations, which might explain the biodegradation results better than a total estimation of the active microbial population. Studies are not yet finished but it can be concluded already that a deeper analysis of microbial diversity is needed and further studies to evaluate in deep the differences observed, should be conducted.

WE076

Impact of erosion of microbial diversity on 2,4-D degradation

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Soil microbes possess vast phylogenetic and functional diversity and are key contributors to pesticide degradation. Functional redundancy, as described in the literature, is observed where species richness declines but ecosystem function is maintained. Little is known about the effects of microbial diversity on soil function under field conditions and even less about the impact of microbial diversity on pesticide degradation kinetics in laboratory studies. This study was conducted to elucidate the importance of microbial diversity in the degradation of 2,4-dichlorophenoxyacetic acid (2,4-D), working towards a model system to assess the sensitivity of pesticide biodegradation kinetics to microbial diversity erosion. To assess the impact of microbial diversity on 2,4-D degradation, mesocosms containing a microbial diversity gradient were generated. Using a dilution to extinction approach, dilutions (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8} and 10^{-10}) of fresh soil were inoculated to soil sterilised by gamma irradiation and autoclaving. After a 26 week equilibration period, analyses to determine microbial biomass (SIR), activity (FDA, DHA) and abundance (qPCR) showed that the mesocosms had reached comparable total biomass. 2,4-D degrader abundance was estimated by most probable number (MPN) analysis. Radiolabelled compound was applied (0.53μg g^{-1} of 2,4-D [ring-^{14}C(U)] to 50g aliquots of soil) and incubated at 20°C in the dark under a flow of moist air. Vessels were sampled after 0, 2, 4, 7, 14, 28, and 60 days, solvent extracted and extracts analysed by LSC and HPLC with radiodetection. Analysis of trapped $^{14}CO_2$ and post-extraction solids was undertaken to obtain a full mass balance. Results from MPN analysis and 2,4-D degradation kinetics showed that erosion of soil microbial diversity up to a 10^{-6} dilution had limited impact on degradation; the remaining 2,4-D degrading microbes being sufficient to maintain this soil function. More severe reductions of overall microbial diversity and of 2,4-D degrader numbers, however, did impact soil function whereby both the half-life and lag phase for 2,4-D degradation increased. Our findings are in accordance with the functional diversity redundancy hypothesis and show our test system as suitable for studying the sensitivity of biodegradation kinetics to microbial diversity erosion for additional pesticides in the remainder of the project.

WE077

Microbial community structure in faecal matter of copepods: Does changes induced by presence of petrogenic oil alter biodegradation?

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The marine copepod *Calanus finmarchicus* is the dominating filter-feeding copepod in the North Atlantic. *C. finmarchicus* has previously been shown to ingest petrogenic oil when exposed to dispersed droplets and ingested oil droplets are excreted together with faecal matter. Faeces from planktonic organisms constitute a significant fraction of sedimenting particles in the marine water column. Since faecal pellets are organically enriched microenvironments, faecal particles are rapidly colonized by smaller organisms, as ciliates and bacteria, and remineralisation of copepod faeces within the mixed water layer is reported to be extensive. The high activity associated with copepod faecal matter is suggested to enhance the biodegradation of oil, and incorporation of droplets in faeces is thus likely to alter fate and transport of dispersed oil. Due to their high abundance and filtering capacity, *C. finmarchicus* may contribute significantly to the overall fate and transport of dispersed oil in the marine environment. We have exposed *C.*

finmarchicus to dispersed petrogenic oil to determine more accurately the significance of the species in fate of dispersed oil in the marine environment. One area of focus has been the microbial community structure associated with copepod faecal matter. Preliminary results from enumeration of bacteria and selective microorganism growth show that microbial communities associated with faecal pellets are altered when copepods are exposed to dispersed oil. Sequencing of DNA extracted from seawater and copepod faecal matter is expected to identify and determine the major changes in microbial communities introduced by the presence of oil droplets. Chemical analysis of the seawater and faecal pellets will identify any changes in the composition of the oil.

WE078

Microbial community capability of PCB degradation in marine sediments

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Microbial communities are responsible for the geochemical cycles and bio-removal of organic compounds and xenobiotics playing a key-role in ecosystem functioning. They are able to adapt promptly to environmental changes and the presence of a natural microbial community is a necessary prerequisite for an effective response to the various chemicals that can contaminate an ecosystem. However, the recovery from contamination is only possible if toxicity does not hamper microbial activity. The knowledge of natural remediation capacity of a microbial community allows assessing the contaminant availability to higher levels in the ecosystem food web. Polychlorinated biphenyls (PCBs) are organic hydrophobic persistent pollutants which are found as diffuse contaminants both in soil and sediment. Bacteria are generally recognized as to be the main responsible of biological processes involved in the aerobic/anaerobic degradation of these pollutants. However, a complete PCB removal from environment still remains a very important issue to be investigated owing to their low bioavailability, co-presence of congeners differing in the number of chlorine atoms attached to the biphenyl rings, etc. In order to assess the natural attenuation capacity of bacterial communities living in seawater sediment, degradation experiments were carried out using PCB-contaminated sediments collected from La Spezia harbor (Italy). The experimental set consisted both of microbiologically active and sterile sediment microcosms, which were maintained over 6 months under aerobic or anaerobic conditions. Chemical and microbiological analyses were periodically performed and allowed to estimate the contaminant degradation kinetics and to evaluate the structure and the composition of the microbial communities selected under each experimental condition.

WE079

Biodegradation of crude oil in adapted and unadapted environments

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Increasing interest in emerging shipping routes and the potential for oil exploitation in Arctic environments has put renewed focus on oil degradation under these specific conditions. Interest in the Arctic region as an oil resource can result in chronic hydrocarbon exposure of this pristine environment, but oil biodegradation is expected to be slow and restricted to metabolically simple oil compounds. Biodegradation in pre-exposed environments, on the other hand, is expected to proceed faster and to encompass metabolically complex compounds, due to adaption of the microbial community in the water column. In this study, we investigated the oil biodegradation potential in waters from two locations, Disko Bay, Western Greenland and the North Sea, Denmark. The Disko Bay is a pristine area of great interest for future oil exploitation. North Sea water from a location 2 km off the coast of Denmark is expected to be pre-exposed to hydrocarbon pollution resulting from shipping traffic and industries. The aim of our study was to evaluate the potential for biodegradation of crude oil in two different environments. This was done by examining the chemical fingerprint of a crude oil, Oseberg Blend, obtained by gas chromatography - mass spectroscopy (GC-MS). Biodegradation of Oseberg Blend crude oil in water samples from the two locations was followed over a 10-week period in microcosms. Changes over time in the microbial oil-degrader populations were determined by MPN enumeration of indigenous bacteria that could grow on hexadecane, m-xylene, 2-methylnaphthalene, 1-naphthol or 1-naphthoic acid. In the adapted North Sea samples, larger concentrations of degrader organisms were present. Initial results suggest different degradation patterns in North Sea samples with degradation of more metabolically complex PAHs compared to Disko Bay samples.

WE080

The microbial potential for crude oil degradation off the coast of Western Greenland is limited.

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In this study, we carried out a qualitative evaluation of the metabolic potential for microbial degradation of oil in water from the Disko Bay and the Baffin Bay (Greenland). Knowledge on intrinsic potentials for oil biodegradation in deep water-bodies in potential oil drilling areas is crucial to predict the fate of oil released in accidental deep-water spills, as biodegradation is the only removal process in deep water bodies. Whereas most previous studies have focused only on alkane removal, BTEX removal or total oil removal, we have instead investigated the potential for microbial growth on 15 model-substrates, and fingerprinting of large oil-compound classes. The selected substrates represented both the alkane fraction (*n*-hexadecane, 2,3-dimethylheptane, cyclodecane, 1,3-dimethylcyclohexane), the monoaromatic fraction (*o*-xylene, *p*-xylene, butylbenzene), the diaromatic fraction (naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 1,3-dimethylnaphthalene) and the polar fraction (benzothiophene, 1-hydroxynaphthalene, 1-naphthoic acid, 1-octanol), and ranged from metabolically simple to metabolically demanding substrates. Water from the Disko Bay (10 and 300 meters depth) and the Baffin Bay (3 and 45 meters depth) were spiked with crude oil, and the evolution of degrader cells able to grow on the model substrates was followed for 90 days. Water from Copenhagen Harbor was included for comparison. The metabolic potential of the Disko Bay and Baffin Bay samples, determined as most probable numbers of specific degraders, turned out to be very limited with growth only on metabolically simple substrates such as hexadecane, butylbenzene and octanol. The Copenhagen Harbor sample, on the other hand, showed degrader populations that were metabolically more diverse, though degraders of the metabolically most demanding compounds were also absent in this sample. The MPN counts were supplemented with a second series of incubations where the most dominant oil degraders from the MPN counts were exposed to crude oil to investigate how these degraders impacted the profiles of *n*-alkanes, branched alkanes, monoaromatics and series of methylated PAHs. All together, these results indicated that the oil degradation potential in the water columns of the pristine Disko Bay and Baffin Bay areas are quite limited, whereas the degrader bacteria from pre-exposed environments may show a much broader substrate range.

WE081

Developing a new saturates biodegradation index for weathered oils, based on samples from the Deepwater Horizon disaster

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After the initial loss of the most volatile and soluble compounds, biodegradation plays a major role in the natural attenuation of oil spills. Monitoring long-term biodegradation of oil spilt in the environment is important, as the remaining compounds can persist for years. However, little quantitative information is available about biodegradation of different saturated hydrocarbon classes in surface environments, despite that crude oils are composed mostly of saturates, and that they remain an important fraction of weathered oils. This is due to the limited ability of conventional gas chromatography (GC) to resolve and precisely quantify different members of this compound group. We studied eight weathered oil field samples collected from Gulf of Mexico beaches 12-19 months after the *Deepwater Horizon* disaster, which led to the release of 0.47 to 0.69 million metric tons of oil into the environment. Using comprehensive two-dimensional gas chromatography (GC×GC), we successfully separated, identified, and quantified several distinct saturates classes from these samples. We found that biodegradation proceeded simultaneously for different saturates classes, but to different relative extents, with ease of biodegradation decreasing in the order: *n*-alkanes > methylalkanes and alkylcycloalkanes > cyclic and acyclic isoprenoids, for compounds in the *n*-C_{22} to *n*-C_{29} elution range. Biodegradation indices, usually expressed as ratios of concentrations of key compound groups, represent a convenient way to quantitatively evaluate the evolution of the oil composition and to link it with the progress of biodegradation. Based on our results, we developed a new saturates biodegradation index designed to characterize weathering of the saturates fraction in field samples, for surface environments. Unlike previously developed biodegradation indices, the new index incorporates information about several classes of saturates, the dominant GC-amenable fraction of moderately weathered oils.

WE082

Atmospheric deposition as a source of bioavailable Polycyclic Aromatic Hydrocarbons for their subsequent biodegradation in the surface global oceans.

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Polycyclic Aromatic Hydrocarbons (PAHs) are organic pollutants generated during

incomplete\ncombustion of fossil fuels and organic matter. They cause carcinogenic and toxic effects in\nbiota, and have been proved to be harmful for ecosystems. Moreover, PAHs are ubiquitous in\nthe environment and show increasing levels in some regions due to growing anthropogenic\nsources. The global ocean has been pointed to hold a strong degradation capacity for these\ncompounds, but it is still necessary to assess the entry and fate of PAHs in the ocean and how\nit is coupled to the biodegradation processes occurring in the surface oceans. Atmospheric\ndeposition is the main vector for the entrance of semivolatile organic compounds to the global\noceans. High MW PAHs are mainly found in the aerosol phase due to their strong sorption to\naerosol soot carbon and low vapor pressure. Therefore, high MW PAHs are mainly settled by\ndry deposition. Conversely, low MW PAHs, the more volatile compounds, are mainly found in\nthe gas-phase, and diffusive air-water exchange is the main process driving their deposition to\nthe surface ocean, supplying bioavailable PAHs to surface waters. Dry deposition fluxes have\nbeen measured, and diffusive fluxes have been estimated from air and water concentrations, for\nall sub- and tropical oceans (35ºN- 40ºS) during the Malaspina 2010 circumnavigation cruise in\nthe Atlantic, Pacific and Indian oceans. Both processes depend on physical factors\n(temperature, wind, humidity, aerosols loadings), chemical properties of PAHs, and\nbiogeochemical controls like biodegradation and the biological pump (processes of\nsequestration in the water column due to adsorption to organic particles and sinking). Those\nbiological processes may be responsible of unbalancing the equilibrium between air and water\nconcentrations, and thus affect PAHs entrance in the ocean; nevertheless few studies have\naddressed them. Here, we show that biodegradation of low MW PAHs by zooplankton and\nbacteria play an important role driving the water column PAH concentrations, while settling\nfluxes towards deep waters plays an important role for high MW PAHs. Therefore a complex\nfeedback is established between depositional fluxes and degradation/bioadsorption processes\nand will be reviewed in this poster. These results are the most extensive data set available for\nthe global ocean and provide evidence of the important physical and biological controls on PAH\noccurrence and cycling in oceanic regions.

WE083

Remarkably rapid dissipation of the herbicide glyphosate in eutrophic Lake Greifensee during summer

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The phosphonate herbicide glyphosate is widely used for weed control in agriculture and forestry, but also in urban areas and can therefore enter rivers and lakes via various pathways. Substantial glyphosate concentrations are regularly measured in surface waters despite its tendency to strong sorption and biodegradation in soils. Once in surface waters, glyphosate levels can be attenuated by several processes including biodegradation. However, detailed knowledge on the behavior of glyphosate in natural water bodies is still limited. In this study, we investigated the occurrence and fate of glyphosate in Lake Greifensee, a eutrophic lake of the Swiss plateau. During the year 2013, monthly depth profiles of the lake, weekly composite samples of the main tributaries, and daily composite samples of a wastewater treatment plant were analyzed for their glyphosate concentrations with an analytical method including derivatization and online-enrichment- LC-MS/MS. A mass balance of glyphosate was then established with a numerical lake model. Glyphosate concentrations in the lake's epilimnion increased from 20 ng/L in March up to 150 ng/L in July due to inputs from the tributaries with concentrations of up to 600 ng/L. Despite elevated input concentrations in July (up to 1000 ng/L), epilimnion concentrations then dropped below the limit of quantification of 5 ng/L in August. This sharp decline was also reflected in glyphosate loads entering and leaving the lake. Inputs of more than 20 kg glyphosate are opposed to an export of only 5 kg via the outflow. Taking the change in lake content into account, more than 10 kg glyphosate dissipated in the lake between March and November. Since photolysis and sorption to particles or sediments are estimated to play a minor role for the loss of glyphosate in the epilimnion, this gap in the mass balance can be largely attributed to biodegradation. Assuming a first-order degradation process in the whole epilimnion, half-lives as low as one day and less were estimated with the lake model. This fast biodegradation coincided with the highest water temperatures, the highest density of phytoplankton and the lowest concentrations of orthophosphate. The latter indicates that glyphosate might have been used as alternative phosphorus source by phytoplankton and/or microorganisms. However, further experiments are foreseen to investigate the interrelationship between changes in microbial communities, nutrient levels, and glyphosate degradation.

WE084

Flume experiments to study the dynamics and mass balance of pharmaceuticals and their key transformation products in the water/sediment environment

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The occurrence and distribution of pharmaceuticals in the environment have raised concern and consequently have been extensively investigated. Nevertheless, understanding of processes that govern their environmental fate is still scarce. Among others, one key limitation is the lack of available data on microbiological transformation processes in the hyporheic zone, where the microbial community is capable of efficiently transforming a variety of pharmaceuticals. More specifically, the link between such processes and hyporheic exchange has not been sufficiently characterized. This knowledge gap is addressed by this contributing study, based on results from experiments with 19 pharmaceuticals in a bench-scale circulating flume that specifically allows studying these process interactions. Surface water and sediment were taken from lake Largen located close to the city of Norrtälje, Sweden. Nineteen pharmaceuticals, covering a wide range of therapeutic classes and physical-chemical properties, were spiked to yield an initial concentration of 10 $\mu g\ L^{-1}$ in the surface water. Experiments were carried out with two different morphologies of the water/sediment interface (even surface vs. artificial ripples) to achieve low and high hyporheic exchange, respectively. Surface water, pore water, and sediment were analysed at up to 8 time points over 30 days. Water samples were filtered and analysed by UHPLC/QqQ-MS, while sediment samples were extracted with accelerated solvent extraction followed by an SPE clean-up before analysis. Both the target pharmaceuticals and their key transformation products were measured. The feasibility of this bench-scale experiment design has been confirmed by the promising results from a pilot study. The formation of 8 out of 11 expected transformation products were detected accompanying the attenuation of parent compounds; their time-trends relative to the parent compounds qualitatively agreed with our results from a transformation product identification study. We will present the mass balance of both the pharmaceuticals and their dominant transformation products in the flume, as well as their elimination/formation kinetics under the two hydraulic conditions. Apart from investigating the fate of pharmaceuticals in this water/sediment environment, we will also discuss the applicability of transformation products as indicators for the elimination of organic micropollutants in the hyporheic zones of rivers and streams.

WE085

Fate of Imidacloprid in soil: Evaluation of various extraction methods and LC-MS analysis of Imidacloprid and its metabolites

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Imidacloprid is a systemic neonicotinoid insecticide with widespread use reaching from seed coating and spray treatment against crop pests and termites to applications in flea treatment for domestic animals. A study has shown that the half-life of the pesticide in soil ranged from 40 days up to 124 days in unamended and manured soil respectively. As far as we know the metabolic fate of imidacloprid in soil has yet not been extensively studied and remains a topic of interest for future risk assessment concerning imidacloprid. This study is part of the project "Evaluation of the risk for soil organisms under real conditions" on behalf of the German Federal Environmental Agency (UBA). We used terrestrial model ecosystems with undisturbed soil cores and analysed the soil treated with radioactively labeled (^{14}C) as well as non-labeled imidacloprid. At different timepoints over the course of 189 days samples in different soil layers up to 20 cm depth were taken and extracted via ultrasonic extraction. The extraction method was compared to microwave assisted extraction, Soxhlet extraction and accelerated solvent extraction. The samples were analysed via TLC, Radio HPLC and HPLC-MS/MS and the metabolites were identified and quantified. The main part of the residues was located in the top 1 cm of the soil, only minor amounts (< 0.1 % of the applied amount) was found in the leachate. Analytical results of the metabolic fate will be presented.

WE086

Fate of lindane and imidacloprid in terrestrial model ecosystems (TMEs) during a sampling period of one year

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A study was conducted in order to correlate the fate and effects of plant protection products (PPP) on soil fauna in different soil layers. Extractable and non-extractable residues of the insecticides lindane and imidacloprid were analyzed and effects on populations of collembola, oribatida, enchytraeidae and earthworms were determined. The analytical part with the spatial and temporal behaviour of the PPP in the soil profile is presented in this poster (the biological part will be presented as platform lecture). The test substances in non-labelled form were applied to TMEs under field conditions and in ^{14}C-labelled form to similar TMEs in the lab. All TMEs (40 cm in height; semi-field: 46.7 cm and lab: 10.0 cm in diameter) contained the same type of undisturbed soil cores with grass cover, and were placed on a perforated plate to collect the leachate. In case of the semi-field study, soils were treated with 7.5 or 20 kg a.i./ha of lindane and 0.75 or 2 kg/ha of

imidacloprid. In the lab study, amounts of 20 kg/ha and 2 kg/ha, respectively were applied using 2.3 MBq of the respective radiolabelled substance per TME. Laboratory TMEs were kept under the same percolation conditions as those in the field (rainfall). Despite different physico-chemical properties of lindane (log Kow > 3) and imidacloprid (log Kow < 1), both substances remained in the top 2.5 cm layer of the soil cores during a period of one year and only small amounts moved into layers below 2.5 cm (6.1 ± 0.7 % of lindane and 18.1 ± 2.7 % of imidacloprid of applied ^{14}C in the lab study). Leachates contained less than 0.1% of the applied amounts of both, lindane and imidacloprid. The portions of non-extractable residues increased to a maximum of 8.9 % and 36.5 % of applied ^{14}C (lindane and imidacloprid, respectively) after 140 days and decreased until 189 days after treatment to to 6.4 % and 24.1 % . The results will be discussed with respect to the relevant soil layer in order to derive the initial predicted environmental concentration (PEC) of the PPPs.

WE087

The influence of the spectral quality of light on the degradation rate of two crop protection products

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Before becoming commercially available, crop protection products (CPPs) must undergo rigorous testing according to regulatory guidelines. OECD guideline 307 dictates that laboratory based experiments used to assess the degradation rates of CPPs in soil are carried out on sieved soil in the dark at constant temperature and moisture. These laboratory experimental parameters do not accurately simulate the inherent variability of the field and consequently, laboratory CPP degradation rates are frequently longer than those found in the field. The soil surface acts as the first environmental point of contact for many CPPs. Previous work showed that the spectral quality of light influences the community composition at the soil surface. Any contribution that phototrophic soil surface communities present in the field may have on CPP degradation rates cannot be evaluated in the current OECD 307 design, which specifies soil should be incubated in the dark. Previous collaborative work between Syngenta and Warwick demonstrated that some CPPs show altered degradation rates when samples were exposed to (non-UV) light:dark cycles, where phototropic soil surface communities were allowed to develop within a modified OECD 307 design. A further experiment was set up to investigate the degradation rates of paclobutrazol and pesticide A under semi-field conditions. Soil cores were placed in semi-field soil plots and pre-incubated for *ca*. 2 months under different light filters, thereby exposing the soil surface communities to differing light regimes. The filters were: clear (all wavelengths transmitted enabling both photosynthesis and photolysis), UV wavelengths removed (>400nm transmitted, enabling photosynthesis only), and photosynthetically active radiation (PAR) & UV removed (450-600nm transmitted, preventing both photosynthesis and photolysis). ^{14}C radio-labelled paclobutrazol or pesticide A were applied to soil cores under each light regime. Soil core samples were taken over time. Each core sample was split into fractions (surface, top bulk and lower bulk) and extracted with organic solvents. The amount of radioactivity in each extract was quantified by LSC. Any unextracted radioactivity present in the soil residue was quantified by sample oxidation and LSC. Soil extracts were analysed by HPLC to determine the amount of parent compound remaining, and parent degradation rates calculated for each light regime.

WE088

Enantioselective degradation of mecoprop in subsoil previously exposed to phenoxy-herbicides

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One fourth of all commercially available herbicides (e.g. mecoprop) are chiral. In the past, they have often been used in 1:1 racemic mixtures. Herbicide enantiomers undergo degradation at different rates in the environment, and therefore the enantiomeric fraction (EF, standard descriptor for molar fraction of R to S enantiomers) progressively deviates from the original value. Due to increasing pesticide contamination of drinking water resources and since the biological effects and toxicity levels have been found to differ among the enantiomers, a more comprehensive understanding of the fate of enantiomers in the environment is necessary. Natural attenuation of mecoprop-contaminated soil was studied by determining enantioselectively the microbial degradation of racemic mecoprop ((RS)-2-(4-chloro-2-methylphenoxy)propionic acid) and identification of metabolites within soil horizons. Enantio-separation of mecoprop was achieved using an enantioselective HPLC column coupled to MS/MS. The results show that mecoprop is degraded with opposite enantio-preference in different subsoil layers. This finding indicates that different microorganisms are responsible for the degradation of mecoprop at 2, 4, and 6 meters depth.

WE089

Comparison of terrestrial field dissipation half-lives of pesticides and their metabolites across Europe

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The normalization of field degradation half-lives to reference conditions for soil moisture and temperature can be used to compensate for the effects of climate on degradation. Through this approach compound degradation from different regions can be compared without being biased by climatic factors to study solely the effects of soil on degradation. Normalizaton has been used in European exposure modeling for several years, however a systematic comparison of degradation across regions has been lacking. In the present study the normalized field degradation of 32 active ingredients and metabolites has been investigated across eight European countries. 170 trials were conducted in northern Europe (Germany, northern France, Sweden, The Netherlands, and United Kingdom) and 75 mainly in the Mediterranean region of southern Europe (Italy, Portugal, southern France, and Spain). Geometric mean half-lives amounted to 35 days (90% confidence interval: 30 days to 41 days) and 43 days (90% confidence interval: 34 to 54 days), respectively, and were not significantly different.

WE090

DEGRADATION OF ATRAZINE APPLIED AS A CONTROLLED RELEASED FORMULATION IN A BRAZILIAN ACRISOL

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The present study evaluated the pattern of atrazine (ATZ) degradation in a controlled release formulation (mineralization, extractable and nonextractable atrazine residues and metabolite formation) in a Brazilian Acrisol, with and without ATZ application over the last 20 years. The results were compared with those obtained in the same samples with soluble atrazine. Xerogel formulations of atrazine (ATZ-XG) were synthesized according to sol-gel method. Incubation experiments were conducted for 85 days with soil samples from an Acrisol (0-10 cm) located in southern Brazil and four treatments were conducted. Microcosm 1 (cultivated soil under no-tillage with crop rotation (maize/soybean) and treated for the past 20 years with ATZ) and Microcosm 2 (native soil collected at an adjacent area with no history of ATZ application) received soluble ^{14}C-ATZ. Microcosm 3 (same soil as Microcosm 1) and Microcosm 4 (same soil as Microcosm 2) received ^{14}C-ATZ-XG. A spiking solution was prepared using technical-grade and 14C-ATZ in ethanol and applied to the Microcosms. The evolved $^{14}CO_2$ and the water and ASE extractable radioactivity were analyzed periodically. The ^{14}C activity was measured with liquid scintillation counter. The cultivated soil that received ^{14}C-ATZ-XG (Microcosm 3) presented a higher ATZ mineralization (93 %) than the native soil (Microcosm 4) (4 %) at the end of the incubation time, and thus corroborating the adaptation of the microflora in the cultivated soil. The water-extractable ^{14}C activity decreased drastically along the incubation in Microcosm 3 (76 to 1%) while that of NER remained around 13%. In Microcosm 4 the decrease of water extractable ^{14}C activity was comparatively smoother (77 to 27%) and a concomitant increase of the ASE (11 to 46%) and of NER ^{14}C-activities (14 to 20%) were observed. In Microcosms 1 and 2 the same trend occurred, but at the end of the incubation the NER ^{14}C-activity was non significant and the ASE ^{14}C activity was around 10 and 50%, respectively. The degradation kinetics was also affected by the formulation type: in the cultivated soil the estimated ATZ half life was 25 days for the ^{14}C-ATZ-XG and 10 days for the soluble ^{14}C-ATZ; in the native soil the calculated values were 963 and 411 days, respectively. The xerogel formulation slowed down atrazine degradation kinetics and promoted its retention in less accessible soil compartments.

WE091

Fate and degradation of Persistent Organic Pollutants (POPs) in the soil of a tropical rainforest

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Soils are crucial reservoirs for the environmentally cycling pool of POPs. Available information on POP fate and degradation in soils almost entirely derives from studies performed in boreal or temperate ecosystems. Tropical and subtropical biomes represent over 60% of total terrestrial productivity and the different conditions compared to those of colder environments may result in markedly different rates of chemical fate processes. Using a set of labelled Polychlorinated Biphenyls (PCBs) added to natural vegetation litter deployed over a structured soil core in a lysimeter, we investigated dynamics of distribution, mobility and

persistence of POPs in the soil of a primary tropical rainforest over a full year period. The endpoints of the study were: i) to assess potential re-volatilization; ii) to measure the rate of the transfer from the litter to the top soil core; iii) to derive degradation half-lives for the litter-soil system; and iv) to describe the dynamics controlling POP mobility and distribution in the soil core. The transfer of POPs from litter to the top of the soil core was the process with the highest rate, and was independent from compound specific properties, suggesting biogeochemical control (namely litter degradation). During the first 4 months of the study volatilization accounted for a loss of about 20 to 30% of the initial spiked mass. After one year, a measurable fraction of all congeners had migrated through the full 10 cm long soil column and was found in the leacheate. Leaching was relatively high for PCB 209 indicating a crucial influence of dissolved organic matter efflux associated transport. Degradation half-lives were estimated to range between 10,000 and 600,000 h and were dependent on chemical properties. These values are about one order of magnitude shorter than those normally used to describe fate in temperate and boreal soils. In contrast to findings obtained from temperate and boreal environments this study showed that POPs can be relatively mobile in heavily weathered tropical soils. Several aspects highlighted here shed lights on process parameterizations which are not considered by the current fate and distribution models. Since tropical environments represent a major fraction of the total land surface area, the implications of these findings may be influencial in the context of the current understanding on POP global distribution and fate.

WE092

Microorganism and Medicago sativa synergic effects on PCB degradation in a contaminated soil

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Polychlorinated biphenyls (PCBs) are organic hydrophobic persistent pollutants which are found as diffuse contaminants both in soil and sediment. Their degradation occurs mainly by biotic aerobic and anaerobic processes mediated by microorganisms. Their degradative activity can be promoted in soil by plant occurrence, because plant roots releasing exudates influence directly and indirectly PCB biodegradation. The effectiveness of PCB degradation is based on the use of toxicant tolerant plant species. Moreover, compost can be added to promote both plant and microbial activity. In order to better investigate the relationships between plant roots and natural microbial populations, soil samples from a PCB contaminated site, localized near Taranto, were used for performing degradation experiments in microcosms. The species *Medicago sativa* and compost derived from municipal solid waste were differently added to soil samples. The experimental set up was maintained in a greenhouse for about 8 months under temperature and water controlled conditions. Microbiological and chemical analysis were carried out at different times (0 , 4 and 8 months) in order to assess the changing in structure and functioning of microbial populations related to PCB degradation. The overall results show the complexity of PCB degradation processes and that each treatment (e.g. plant occurrence/absence or presence/absence of compost) acts differently on the degradation of the various PCBs analyzed, promoting the decrease of some congeners and the formation and accumulation of others.

WE093

Plant-assisted bioremediation as a green technology for recovering soil from PCB contamination

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Bioremediation is an increasingly popular alternative to conventional methods for treating waste compounds, in line with environmental sustainability, with the possibility to degrade contaminants using natural microbial activity mediated by different consortia of microbial strains. Among the techniques of so-called green remediation, plant-assisted bioremediation seems to be one of the most promising techniques. Plant-assisted bioremediation is a technology that exploits the synergistic actions that are established in the rhizosphere between plant roots and microorganisms in order to remove, transform or immobilize toxic substances. The presence of plant species through the roots promotes the modification of the physico-chemical properties of contaminated soils as well as the release of root exudates. Organic pollutants may be partially co-metabolic degraded by root enzymes and/or completely biodegraded by microorganisms in the rhizosphere. The phytoremediation effectiveness depend on the use of plant species tolerant to the toxic effects of contaminants in the soil and with roots able to promote the development of a microbial community capable of supporting the degradation of the contaminant in the rhizosphere. This technique is used in preliminary experiments, one in the field and in two in greenhouse, using a soil diffusely contaminated by PCBs in which different plant species were tested. In the field experiment, two tree species (Tamarix gallica and clone Monviso of the genus Populus), were used. At the same time, soil microcosms were set up in greenhouse in order to study in detail the possible PCB degradation processes that occur in the rhizosphere. For this purpose, some microcosms were prepared in the presence/absence of Medicago sativa specie and others in the presence of the Monviso clone, the same used in the field experiment. In this work we describe the various experiments and their main results.

WE094

Degradation of 3- and 4-ring azaarenes by bacterial isolates from soil

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Azaarenes are constituents of coal tar and often found as environmental pollutants. However, very little is known about which bacteria have the ability to degrade these compounds. However, often the toxic potential of the coal tar spills is rather related to the heterocyclic than the hydrocarbon based PAH. A number of soil-derived bacterial degraders of polycyclic aromatic compounds (PAC) were investigated for their capacity to metabolize 3- and 4-ring azaarenes. In resting cell assays, pure cultures of Sphingomonas paucimobilis (strain EPA505), Ralstonia eutropha, Mycobacterium frederiksbergense, Variovorax paradoxus, Microbacterium sp., Dyatobacter fermentans, Burkholderia sp., Pseudomonas frederiksbergensis and others were tested for degradation of, and metabolite formation from non-labeled N-substituted PAC. Substrates were added at 300 (4-ring azaarenes) or 5000 (3-ring azaarenes) microgram/L of liquid minimal medium and included the 3-ring compounds acridine ($C_{13}H_9N$), phenanzine ($C_{12}H_8N$), phenanthridine($C_{13}H_9N$), 5,6-benzoquinoline ($C_{13}H_9N$), 7,8-benzoquinoline ($C_{13}H_9N$) , and the 4-ring compounds benz[a]acridine ($C_{17}H_{11}N$), benz[c]acridine ($C_{17}H_{11}N$), dibenzo[f,h]quinolone ($C_{17}H_{11}N$). Analysis of residual substrates and metabolites formed was performed using HPLC-MS/MS. The results indicated towards different degradation routes: introduction of hydroxy groups to the aromatic backbone was observed as well as addition of water (under reduction of an aromatic double bond to a single bond) as well as addition of two hydroxyl groups at the same time. However several compounds proved to be recalcitrant against all bacteria tested in resting cell assays. Three-ring systems could be degraded by 17 out of 18 tested isolates. Four-ring azaarenes could be degraded by all isolates except Microbacterium sp. and one of the Mycobacterium spp. However, the degradation rates and the metabolites formed varied a great deal between the individual isolates and compounds. Among the 4-ring compounds, the degradability decreased from Dibenzo[f,h]quinoline over Benz[c]acridine to Benz[a]acridine. On the poster an overview on the degradability of the single compounds by the various bacterial isolates will be given.

WE095

The degradation efficiency of oil properties of Rhodococcus sp. Y2-2 at low temperature, isolated from oil-contaminated soil in South Korea

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Rhodococcus sp. Y2-2 isolated from oil-contaminated soil by using a newly developed transwell plate method has proved to be one of most effective strains for treating oil-contaminated soil during winter in large-scale area. It grows well at around 10ºC in mineral salt medium (MSM) at various concentration of oil: 1000; 1500; 2000; 5000; 10000; 20000 and 50000 ppm (w/w) oil (equal concentration for each kerosene, gasoline and diesel). Its degradation efficiency was about 84.48% during 2 weeks. To get the optimum degradation conditions, the pH test was performed in the range of pH 5 to pH 10. Temperature effect was tested at 4; 10; 15; 20; 28; 37 and 40ºC. Inoculum size ranging from 0.5 g-wet cell/L to 10 g-wet cell/L were used to determine the degradation rate at each concentration value of oil. The optimum rate was occurred at pH 7 and 0.5 g/L inoculum size at oil conc. 1500 ppm. Therefore, *Rhodococcus*sp. Y2-2 may be a good biological source for bioremediation in oil-contaminated soil especially during cold winter season.(This study was supported by the GAIA project (2012000550024) funded by Korea Environmental Industry & Technology Institute and Korean Ministry of Environment.)

WE096

Mineralisation and biodegradation of aromatic organophosphorus flame retardants

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Several organophosphorus flame retardants (OPFRs) have been proposed as potential replacements for brominated flame retardants in polymers, textiles and electronics, although the PBT properties of many of these alternative flame retardants are poorly characterized. As part of the EU FP7 ENFIRO project, we therefore determined the aerobic biodegradability of the selected aromatic OPFRs

(triphenylphosphate (TPP), resorcinol bis(diphenylphosphate) (RDP), bisphenol-a bis(diphenylphosphate) (BDP) and 9,10-dihydro-9,10-oxaphosphaphenanthrene (DOPO)) in a study based on OECD guideline 301 with diluted waste water treatment sludge. Mineralisation of the OPFRs was followed by quantifying the release of carbon dioxide. Primary biodegradation was followed by LC-MS/MS analysis of the flame retardants and transformation products were identified using LC-HRMS. The results show that only TPP is mineralised rapidly enough to be classified as readily biodegradable. The rates of primary biodegradation of the OPFRs varied, with TPP, RDP and DOPO being removed to undetectable levels in less than 14 days. In contrast, no significant removal was observed for BDP. These results indicate that as far as persistence is concerned, BDP is less suitable than TPP, RDP and DOPO as an alternative flame retardant. However, a full evaluation of the environmental safety of these OPFRs also requires consideration of their toxicity, technical performance and life cycle impact.

WE097

Biological treatment of micropollutants in drinking water resources

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Contamination of drinking water resources is becoming a threat that is particularly widespread. Nowadays even in European countries clean drinking water is at risk. Pharmaceuticals, pesticides and other micropollutants are emerging substances in surface and groundwater causing contamination of drinking water resources and ultimately to closing down groundwater abstraction wells. Closure of the groundwater abstraction wells or the entailing treatment is costly. It is important to find a sustainable and cost-effective remediation technique since it remains unknown if there are long term cumulative dose-additive or synergistic effects of low concentrations of substances occurring as a mixture. Knowledge of degradation processes at such low concentrations is limited and novel approaches are needed to develop biological treatment technologies that are efficient at these low concentrations. BIOTREAT is a European project in which *urgently needed sustainable biotechnologies* are developed for remediation of drinking water resources contaminated with micropollutants such as pesticides and pharmaceuticals and their metabolites. Many micropollutants are exceeding EU standards. The compound BAM has been chosen as model compound for metabolic biological degradation. 2,6 Dichlorobenzamide or BAM is a metabolite of the broadly used herbicide dichlorobenzonitrile or dichlobenil. The bacterium *Aminobacter* sp. MSH1 is found to be capable to mineralize BAM. In order to simulate drinking water production at waterworks we have up scaled the lab-scale batch experiments to a sand filter column experiment in our lab and finally to a medium scale sand filter column that is used as experimental column at a drinking water well. The BAM mineralizing bacterium was found capable to degrade the metabolite in batch culture and sandfilter column experiments at low concentrations. Pilot scale sand columns have been designed based on these results.

WE098

Determination of transport properties and biodegradation of acesulfame in laboratory column experiments, batch studies, and a fixed-bed reactor

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Acesulfame, an artificial sweetener, is widely used, e.g. in beverages and low-calorie food. After consumption, acesulfame is excreted and ends up in surface waters due to its high stability during waste water treatment. Concentrations up to 10 μg/L have been reported for smaller streams, while in big rivers like the Rhine, concentrations range from approximately 0.5 to 3 μg/L. These comparatively high concentrations in surface and wastewater combined with common and easily adaptable methods for determination like SPE followed by HPLC-MS/MS make acesulfame a potential tracer for a wide range of environmental applications. Despite the ongoing discussion and de-facto growing use of acesulfame as a tracer, information on its transport properties and potential biodegradation in the environment is scarce. To close the gap of knowledge, biodegradation of acesulfame was tested in different aqueous matrices in a fixed-bed reactor and sorption was determined in batch experiments according to modified OECD guideline. Moreover, reactive transport was studied under different redox settings in flow through laboratory columns filled with sediments from a bank filtration site. Columns were operated for 7 month to allow adaptation of the microbial community and to avoid short-term effects. Results of batch experiments yielded low sorption for several soils and Kd was < 0.1 cm^3/g. Biodegradation at environmental concentrations of 9 μg/L was not observed in several aqueous matrices, except for diluted effluent of a waste water treatment plant. In this matrix biodegradation started after 17 days of adaptation. The latter is surprising and puts reasonable doubt on the assumption that acesulfame behaves in general conservative in the environment. However, flow-through column experiments did not indicate non-conservative behavior, as recovery of added acesulfame exceeded 90 % and the retention time was clearly below 1 day.

WE099

Removal of estrone with biogenic manganese oxide nanoparticles

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Conventional wastewater treatment technologies often do not remove pharmaceuticals efficiently. Consequently, pharmaceuticals find their way into the aquatic environment as micro-pollutants. Biogenic manganese oxides nanoparticles (BioMnOx) appeared as an attractive alternative to remove otherwise persistent micro-pollutants. Implementation of BioMnOx in wastewater treatment requires that Mn^{2+} oxidation and pollutant removal by BioMnOx can occur *in situ*, but little is known about the potential of BioMnOx to remove a wide range of micro-pollutants under *in situ* conditions. Here we present the reactivity of BioMnOx, produced by *Pseudomonas putida in situ* (including growth media), towards a wide range of micro-pollutants at low concentrations (10 $\mu g\ L^{-1}$). We found that a steroid hormone, estrone, was readily removed with BioMnOx within the first 33 h after the addition, while no removal occurred in the bacteria free and manganese free controls during 300 h of incubation. The process needs to be optimized to remove ibuprofen, tebuconazole and diclofenac. Our results point toward the prospective of BioMnOx in advanced water treatment.

WE100

Biodegradation of Nitrosamines and Nitramines under Anoxic Conditions

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Among the large number of degradation products that may form in amine-based post combustion CO_2 capture (PCCC) plants, nitramines (R_2NNO_2) and nitrosamines (R_2NNO) are of concern due to their carcinogenic potency. Once released into the atmosphere, these highly water soluble compounds may undergo wet deposition to aquatic and terrestrial environments. To assess the risk regarding the release of these chemicals, it is important to understand how their environmental persistency and accumulation is influenced by processes such as photolysis, soil adsorption and biodegradation. The work reported here is part of a larger study investigating the environmental fate of nitrosamines and nitramines relevant to PCCC. Previous studies have shown both nitrosamines and nitramines are resistant to aqueous hydrolysis and that nitrosamines, but not nitramines, rapidly photodegrade in natural sunlight. Freshwater biodegradation of these compounds has been shown to be dependent on the presence of other functional groups (e.g. hydroxyl) in the molecules and analyte concentration. In the current study the anoxic biodegradation rate of two nitramines (2-nitroaminoethanol, MEA-NO_2; dimethylnitramine, DMNA) and two nitrosamines (nitrosodiethanolamine, NDELA; nitrosodimethylamine, NDMA) in lake sediment is determined and related to the degradation rates observed in freshwater under oxic conditions. Two of the compounds (NDELA and MEA-NO_2) have previously been shown to biodegrade in freshwater, whilst two (NDMA and DMNA) exhibited significant resistance. The biodegradability under anoxic conditions in lake water sediment was studied over a period of two months.Comparisons between microbial degradation rates determined under anoxic (sediment) and oxic (freshwater; existing data) conditions show similar patterns, with compounds containing hydroxyl group more susceptible to biodegradation. As part of this study, the adsorption kinetics of the nitrosamine and nitramine compounds to four natural soil types collected around Trondheim (Norway) was investigated according to OECD Guideline 106. Under sterile conditions no adsorption was observed to any of soils types for any of the compounds. However, when the soil was not sterilized, a significant depletion was observed for several of the compounds after only 48 hr exposure. This indicates rapid aerobic degradation by soil microorganisms, but interestingly a different pattern of degradation was observed compared to aqueous media.

WE101

The fate of fluoxetine under iron- and sulfate-reducing conditions

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Fluoxetine is a highly consumed antidepressant. In Germany, the amount of prescribed fluoxetine was 1.1 tons per year in 2012. [1] After administering, fluoxetine and its human metabolites are excreted via urine and reach the wastewater treatment plants (WWTPs). Fluoxetine is known to pass municipal WWTPs and is discharged into surface waters. Hence, typical biological aerobic/anoxic treatment processes of WWTPs are usually not sufficient for its removal [2] This is of particular concern for the aquatic environment, since it is a neuroendocrine disruptor. Even if the levels of chronic and acute toxicity are relatively high, sub-lethal effects on aquatic organisms have been shown to occur even at concentrations in the ng/L-range [3]. The current study aims to search for

alternative biological treatment options using batch experiments under strictly anaerobic redox conditions and to identify anaerobic transformation products (TPs). Iron- as well as sulfate-reducing conditions were found to be suitable for the degradation of fluoxetine in anaerobic batch experiments conducted with activated sludge incubated under an argon atmosphere. The analysis by liquid chromatography coupled to high resolution mass spectrometry using LC-Orbitrap MS revealed the formation of one anaerobic TP, which was tentatively identified by a combination of MS fragmentation experiments and application of chemical reactions (e.g. ester hydrolysis). The proposed chemical structure of the TP indicates a multi-step transformation including the methylation of an aromatic ring system, β-cleavage of the amino group and a complex rearrangement. Currently, the TP is isolated for a final confirmation of its structure by NMR experiments and for the development of a sensitive LC-MS/MS method for quantification. The quantification method will be used to assess mass balances and to elucidate whether the results can be also transferred to a larger scale of an anaerobic pilot plant.

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WE102

Transformation of veterinary pharmaceuticals and biocides in (liquid) manure – International validation ring test

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Veterinary pharmaceuticals (VMPs) administered to animals are excreted with urine and feces. For animals housed in stables the resulting manure is collected and stored before being spread onto agricultural land. Disinfection products used to sanitize stables are also transferred into the manure. Therefore, the spreading of manure is an important pathway of introducing VMPs, biocides and their metabolites and transformation products into the environment. As a consequence, the fate of VMPs/biocides in manure is taken into account in the environmental risk assessment for VMPs/biocides. Although there is the need for guidance on the performance and evaluation of degradation studies with VMPs/biocides in manure, a standardized and validated method is currently lacking. Indeed, the European Medicines Agency (EMA) adopted a guidance document on determining the fate of VMPs in manure in March 2011. However, the document is intended to provide guidance on the general conditions of studies on the transformation of VMPs in manure, but is not an experimental protocol. Thus, further advice on experimental details is required to obtain reliable and sound results. Against this background, a standardised experimental test method and a draft guideline are currently under development within a research project funded by the German Federal Environment Agency that should in the long run lead to a guideline on transformation of substances in (liquid) manure. To test the applicability of the draft test method an international inter-laboratory comparison (pre-validation ring test) has been performed in 2012/2013. Based on the results and experiences, the test method has been improved and the draft guideline has been revised. Currently, the test method is being validated in an international ring-test by seven institutes from Europe and Northern America. The anaerobic transformation of two ^{14}C-labelled compounds is examined over an incubation period of 90 days (one VMP in pig manure, one biocide in cattle manure). Besides a ^{14}C mass balance, the following endpoints/parameters are determined, if possible: disappearance time (DT_{50}) of the parant compound; formation of transformation products; mineralisation (CO_2 + CH_4); extractable residues; formation of non-extractable residues (NER). This poster describes the test method used within the international validation ring test and presents first results.

WE103

Draft OECD test guidance for transformation of veterinary pharmaceuticals and biocides in liquid manure – robustness test

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Currently, neither an EU- nor an OECD-test guideline exists to experimentally determine the transformation of veterinary medicinal products (VMP) and biocides in liquid manure. For both VMPs and biocides, manure application onto agriculturally used soils is supposed to be the main path of entry into the environment. Thus, respective guidance documents – such as "Guideline on determining the fate of veterinary medicinal products in manure" (EMA/CVMP/ERA/430327/2009) – demand respective experimental tests and give a rough frame for it. As the results of such test will be used for registration, the studies have to follow a harmonized internationally accepted method. As well known from other legislative frameworks, a test guideline is needed which detailed describes the experimental procedures while taking into account the specifics of the complex matrix "liquid manure". The R&D-project „development of test guidance for transformation of veterinary pharmaceuticals and biocides in liquid manure" (UBA FKZ 3710 67 422) resulted – starting with existing OECD-test guidelines such as OECD 307, OECD 308 and OECD 309 – in a draft guideline for experimental setup and performance. The draft guideline was in a first step approved by statistical analyses and by an intra- and an inter-laboratory comparison. However, the influence of some experimental details on the result of a transformation study in liquid manure still remained unsolved. Thus, a second research project was initiated by the German Federal Environmental Agency to experimentally prove or disapprove the validity of the suggested method. In particular, the influence of various parameters on the mineralization rate – expressed as CO_2- and CH_4-formation and on the CH_4/CO_2-ratio is addressed in the current study. The robustness of the method against the following parameters are investigated: comparison of flow-through system versus static system; influence of the flow rate; size of individual subsamples; dry matter content of the manure. Beside the formation of CO_2 and CH_4, the extractables, the non-extractable residues (NER) and the recovery at the end of the transformation studies were checked for each of the tested variations and both cattle and pig manure. Data determined so far in the laboratory experiments demonstrate that the draft guideline represents a pretty robust test procedure already.

WE104

Transformation of veterinary medicines in liquid manure – A literature study

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Veterinary medicinal products (VMPs) enter the environment via manure application. This application practice constitutes a potential environmental risk. During manure storage transformation processes of VMPs take place. Within a literature study the following questions were raised: What kind of compounds are investigated in previous transformation studies, what kind of methods and analytics are used and what are the important factors which affect the degradation and transformation process in liquid manure? For this purpose research articles and reports about transformation of VMPs in swine and cattle manure have been evaluated. Most of the veterinary medicines used in livestock are antibiotics like tetracyclines, sulfonamides and macrolides. Transformation of these active agents may occur by abiotic processes like hydrolysis or photo degradation and by biotic (enzymatic) reactions. Transformation products of VMPs and their metabolites are also capable to persist in environmental matrices and can be of ecotoxicologic relevance. Most research articles describe LC-MS/MS as the analytical method for detection and identification of parent compounds and metabolites. The use of 14C-radioactive test compounds is a appropriate method to differentiate between degradation and formation of non-extractable residues. Transformation processes are influenced by the composition of matrix, temperature, and pH-value. Further, transformation rates differ whether aerobic or anaerobic conditions are prevailing. Compounds adsorb to the matrix depending on the sorption capacity and chemical-physical properties of the test compounds. During the manure storage in manure tanks, mostly applied in Europe, the storage conditions are anaerobic. Composting the separated solid manure under aerobic conditions stimulates degradation of compounds. Concluding, the transformation process of compounds is affected considerably by the storage practice of manure.

WE105

Dissipation of the antiparasitic agent ivermectin in cattle dung and soil at 4 parallel field studies

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The macrocyclic lactone ivermectin is one of the world's most widely used antiparasitic medicines for the treatment of farm and domestic animals. After treatment of cattle, up to 80 % of the drug is excreted unchanged via faeces. A field study was performed with cattle dung in four different ecological regions in Europe (Switzerland, Netherlands, France) and Canada. Herewith, the influence of different climatic conditions on ivermectin dissipation in cattle dung and on the occurrence of ivermectin in soil below dung pats was assessed. Dung of treated cattle with different initial concentrations was stored in the field for up to 12 months. At one field site (France) spiked samples were in parallel exposed in the

field. The analytical method for determination of ivermectin in soil and dung consists of a simplified robust acetonitrile extraction, in which there was no need for a time consuming sample purification. It was shown that before extraction re-wetting of the samples to uniform water content is necessary in order to avoid analytical artifacts. The subsequent HPLC-fluorescence detection after a specific derivatisation was used as a robust, selective and sensitive instrumental method. For soil samples a limit of detection (LOD) of 0.9 μg / kg_{dw} and a limit of quantification (LOQ) of 2.3 μg / kg_{dw} were determined. The LOD for dung samples was 5.1 μg / kg_{dw} and the LOQ 12.4 μg / kg_{dw}. The mean recovery of the internal standard doramectin was 97.7 % (RSD 10.1 %) for the soil samples and 99.1 % (RSD 10.2 %) for the dung samples exposed in the field. Ivermectin concentrations in soil did not exceed 36 μg / kg_{dw} at month three after starting the study. At month thirteen ivermectin was still detectable in soil of the higher treatment levels. It was shown that there is a need to bring dung samples exposed in the field to uniform water contents before extraction, which queries past results for half-lives. Differences in dissipation characteristics for different sampling sites were shown.

WE106

Accelerated Biodegradation of Antibiotics in Agricultural Soils Following Exposure in the Field

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Antibiotics can be entrained into agricultural soil via fertilization with manures or sewage sludge that contain excreted drug residues. In order to evaluate the fate and effects of antibiotics in soil, in 1999 we initiated on our research farm in London, Ontario a long term field experiment. Every subsequent spring, a series of small plots have received an annual spring application of a mixture of tylosin (Tyl), sulfamethazine (SMZ) and chlortetracycline (CTC) to achieve concentrations (mg/kg soil) of 0.1, 1, or 10. In 2010 several more plots were established that received an annual spring application of a mixture of erythromycin (ERY), clarithromycin (CLA) and azithromycin (AZI) to reach concentrations of 0.1 or 10 mg/kg soil. Control plots are maintained that do not receive antibiotics, and all plots were continuously cropped to soybeans. The soil persistence and dissipation pathways of the antibiotics are evaluated in the laboratory using radioisotope methods and HPLC-MS. Compared to soil with no history of drug exposure, the persistence of TYL, SMZ, ERY and CLA was far shorter in soils that were exposed to the antibiotics in the field. ^{14}C-labelled SMZ, ERY and CLA were rapidly and thoroughly mineralized by the exposed soil, whereas not at all in the unexposed control soil. Enhanced degradation of ERY and CLA was established with exposure to both 0.1 and 10 mg/kg. Enhanced degradation of SMZ was detected in soils exposed to 10 mg/kg, but not lower concentrations. The relationship between abundance of SMZ-degrading bacteria, SMZ concentration, and mineralization of the drug was explored in laboratory incubations with an SMZ-degrading bacterium, *Microbacterium* sp. Strain C448. There was insignificant mineralization of ^{14}C-SMZ in uninoculated soil amended with SMZ concentrations from 0.01 to 10 μg/g. In soil inoculated with 10^7 viable C448/g, ^{14}C-SMZ was mineralized with comparable kinetics regardless of the drug concentration. However, in soil inoculated with 10^4 viable C448/g, there was very little mineralization at concentrations of 0.01 to 1 μg/g SMZ, whereas at a concentration of 10 μg/g, the rate of $^{14}CO_2$ accumulation was very significant. These results are consistent with enhanced biodegradation of a variety of antibiotics that vary widely in structure. We hypothesize that repeated exposure to drug concentrations sufficient to support growth in soil is required for the development of a biodegrading flora.

WE107

Tracking the uptake and metabolism of munitions compounds in coastal marine ecosystems using stable isotopic tracers: Role of sediment

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It has been estimated that there are hundreds of explosive-contaminated sites all over the world and managing these contaminated sites is an international problem. As sediments are often a sink for these compounds, it is important to understand their role in remediation. This study uses stable isotopes to track the metabolic pathways of these trinitrotoluene (TNT) and trinitrotriazine (RDX) in sediments. Two aquaria scale experiments were conducted separately for TNT and RDX. Experimental tanks were loaded with sediment and biota prior to the introduction of labeled compounds to the tanks. The initial pulses of compounds were followed by subsequent additions in an effort to maintain a constant concentration in the tanks. Sediment, pore-water and overlying water samples were analyzed for parent and degradation products. Isotope analysis of the bulk sediments revealed a rising inventory of ^{15}N illustrating the role of sediments on sorption and degradation of both compounds. Higher sediment ^{15}N enrichments were found for the TNT treatment than for RDX showing that TNT and its derivatives had a higher affinity for sediment than RDX and its derivatives. Pore-water samples were analyzed for ^{15}N inventories in different pools of nitrogen including ammonium, nitrate, nitrite and nitrogen gas. The ^{15}N tracer was found in pools for both compounds illustrating that mineralization of both compounds occurred in sediments. The results will be used to identify the mineralization pathways of parent compounds in sediments. Ultimate denitrification rates for TNT and RDX in pore-water are 0.02 µmolhr^{-1}and 0.64 µmolhr^{-1} respectively and were a significantly smaller than ^{15}N accumulation rates in sediments. These experiments will be extended into 500 gallon ecocosms with biota to address the importance of sediments on mineralization of explosives in large scale. Chemical parameters including pH, salinity, dissolved oxygen, dissolved organic matter, iron and sulfide in sediment profiles correlated to explosive metabolism will be evaluated.

WE108

Climate change effects on PAH photodegradation in Mediterranean soils: A pilot study

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Climate change is one of the most important problems to be faced by the mankind in the 21th century. It is estimated that this phenomenon may cause notorious environmental changes around the world. More specifically, one of the consequences of climate change that has recently attracted most attention is its potential to alter the environmental distribution and biological effects of chemical toxicants, therefore having a significant impact on human health. In recent years, persistent organic pollutants (POPs) have drawn scientific and political concern. Although not listed as POPs in the Stockholm Convention, some polycyclic aromatic hydrocarbons (PAHs) are persistent in the environment, bioaccumulative, highly toxic, and capable to be transported over long distances from the emission sources. Given this, the 1998 Aarhus Protocol identified four PAHs as POPs: benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-cd]pyrene. It has been demonstrated that variables such as temperature and solar radiation may notably influence the environmental fate and distribution of POPs in general, and PAHs in particular. This study aims to estimate the degradation of 16 PAHs in Mediterranean soils as a consequence of variations in temperature and UV-B radiation according to different IPCC climate change scenarios estimated for the Mediterranean basin. This area is considered one of the most vulnerable regions of the world to climate change, as it is likely to be affected by interactions between mid-latitude and tropical processes. Cleaned soil samples were spiked with 16 PAHs and exposed to different simulated scenarios of temperature and UV-B radiation in a controlled climatic chamber. The results showed variations in the PAH concentrations according to the molecular weight, presenting naphthalene the highest photodegradation rate. Our findings highlight the clear impact of climate change on the fate and behavior of PAHs, and the potential changes in the human health risks associated to PAH exposure. Further studies will have to elucidate the potential formation of metabolites in the PAH photodegradation process, since some of them could be even more toxic than their parental compounds.

WE109

phytotreatment of polychlorinated biphenyls contaminated soil by Chromolaena odorata (L) King and Robinson

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The ability of *Chromolaena odorata* propagated by stem cuttings and grown for six weeks in the greenhouse to thrive in soil containing different concentrations of PCB congeners found in Aroclor 1254, and to possibly remediate such soil was studied under greenhouse conditions. *Chromolaena odorata* plants were transplanted into soil containing 100, 200, and 500 ppm of Aroclor in 1L pots. The experiments were watered daily at 70 % moisture field capacity. Parameters such as fully expanded leaves per plant, shoot length, leaf chlorophyll content as well as root length at harvest were measured. PCB was not phytotoxic to *C. odorata* growth but plants in the 500 ppm treatment only showed diminished growth at the sixth week. Percentage increases in height of plant were 45.9, 39.4 and 40.0 for 100, 200 and 500 ppm treatments respectively. Such decreases were observed in the leaf numbers, root length and leaf chlorophyll concentration. The control sample showed 48.3 % increase in plant height which was not significant from the treated samples, an indication that *C. odorata* could survive such PCB concentration and could be used to remediate contaminated soil. Mean total PCB absorbed by *C. odorata* plant was between 6.40 and 64.60 ppm per kilogram of soil, leading to percentage PCB absorption of 0.03 and 17.03 % per kilogram of contaminated soil. PCBs were found mostly in the root tissues of the plants, and the Bioaccumulation factor were between 0.006-0.38. Total PCB absorbed by the plant increases as the concentration of the compound is increased. With these high BAF ensured, *C. odorata* could serve as a promising candidate plant in phytoextraction of PCB from a PCB-contaminated soil. ***Keyword***: Phytoremediation, Bioremediation, Soil restoration, Polychlorinated biphenyls (PCB), Biological treatment, Aroclor.

WE110

In-vitro study of acyl-transferase activity of Rhodococcus and Bacillus

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With *Rhodococcus* maximum acyltransferase activity was obtained in 100mM glycine-NaOH buffer (pH 8.5) with substrate concentration 0.850 mmoles of acetamide and 1.7 mmoles of hydroxylamine hydrochloride with resting cells 0.94 mg (cell dry weight) for 20 minutes at 45^{O}C. It had showed broad substrate specificity. Propionamide was the best substrate followed by butyramide, acetamide and lactamide. Among the various metal ions and inhibitors, $CuSO_4.5H_2O$ had severely affected the enzyme activity. The enzyme was fairly stable at 4^{O}C and room temperature. Degradation of acetamide for 5 h occurred in the range of 70 to 80% while in case of *Bacillus* maximum acyltransferase activity was obtained in 100mM potassium phosphate buffer (pH 7.5) with substrate concentration 0.850 mmoles of acetamide and 1.7 mmoles of hydroxylamine hydrochloride with resting cells 0.94 mg (cell dry weight) (0.322 Uml^{-1}) for 20 minutes at 55^{O}C. It had showed broad substrate specificity. Acetamide was the best substrate followed by acetonitrile. The higher relative activity against acetamide proved that this reaction was catalysed by two enzymes (i.e. nitrile hydratase and amidase) and amidase, which catalyse acetamides hydrolysis, had more activity in comparison to nitrile hydratase of acetonitrile hydrolysis pathway. Therefore, nitrile hydratase was a step regulating enzyme. Among the various metal ions and inhibitors, $AgNO_3$ had severely affected the enzyme activity. The enzyme was fairly stable at 4^{O}C and room temperature. Degradation of acetamide for 5 h occurred in the range of 80 to 90%. This amido transferase activity has potential applications in enzymatic synthesis of hydroxamic acids and bioremediation of nitriles and amides contaminated soil and water system. **Keywords:** Acyltransferase, Hydroxamic acid, *Bacillus, Rhodococcus*, Acetamide, Bioremediation

Identification and prioritisation of hazardous emerging pollutants (P)

WE111

Applicability of existing sequential extraction schemes to identify the fractionation of fluorine in soil

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Understanding the fractionation of elements in soil is so significant because it makes site-specific assessment of bioavailability and reliable risk to human and environment. Sequential chemical extraction (SCE) is one of the prominent tools to estimate the partitioning states of particulate trace elements. While the large number of studies has dealt SCE schemes of metal cations, only limited works have been doned for anions such as fluorine (F). Although F compounds in soil and groundwater draw public attention due to their potential toxicity including crippling bone diseases, fractionation scheme of F has been not standardized yet. However, the operationly defined chemical form of some schemes have similarities with natural fate of F in literatures.\nIn this study, we compare three existing SCE schemes to investigate their applicability for F. Two schemes are typical ones for metal cations by Tessier et al. and arsenic by Wenzel et al.. And the other one from Xu et al. is suggested for sequential extraction of F. Despite Wenzel et al. also reported the scheme for F, it was not considered here for its similarity with Tessier et al.. The total F concentration for each sample was assessed according to the Korean official test methods of soil, and recovery rate throughout steps will be a yardstick for estimating applicability. To figure out the combined cations, all of the supernatant liquids including washings are analyzed by ICP-AES.\nThe aims of this study are to establish the SCE scheme and to identify the environmental fate of F in soil. The information on fate of F is expected to be useful for the management of F leakage from industrial facilities. This presentation will include the up-to-date results. References\nTessier A, Campbell PGC, Bisson M. 1997. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 51: 844-851.\nWenzel WW., Blum WEH. 1992. Flurine speciation and mobility in F contaminated soils, Soil Sciences 153: 357-364.\nWenzel WW, Kirchbaumer N, Prohaska T, Stingeder G, Lombi E, Adriano DC. 2001. Arsenic fractionation in soils using an improved sequential extraction procedure. Analytica Chimica Acta 436: 309-323. \nXu L, Luo K, Feng F, Tan J. 2006. Flurine content and distribution pattern in Chinese coals. Fluoride 39: 145-151. Acknowledgement - This research was supported by a grant from the KBSI (Project No. C3372A) and Geo-Advanced Innovative Action Project by the Korean Ministry of Environment.

WE112

Optimization of ultrasound assisted enzymatic extraction for the analysis of fluorine contents in rice samples

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An alkali fusion-selective ion electrode is generally used to determinate the fluorine content in soil and vegetation samples [1]. Nevertheless, the extraction procedure is complex and time consuming. In order to simplify the extraction procedure and reduce the extraction time for determination of fluorine content, sonication process was applied with enzymes such as α-amylase and protease. The extraction procedure of ultrasound assisted enzymatic extraction was described in E. Sanz et al. [2] for analysis of arsenic in plant samples. The experimental result of ultrasound assisted enzymatic extraction was compared with that of alkali fusion extraction method to validate the accuracy of proposed method. And the optimizatioin of the extraction of fluorine content in rice samples is necessary to establish a rapid and accurate analysis. Therefore, the factors affecting the extraction efficiency such as sonication time, the mass of α-amylase and protease, the depth of sonication probe, and solvents were evaluted to determine the optimum condtions of proposed method. In this study, we evaluated the ultrasound assisted enzymatic extraction method in rice samples and determined the optimum conditions of proposed method. This study received substantial support from the Geo-Advanced Innovation Action (GAIA) project of the Korea Environmental Industry & Technology Institute (KEITI). The authors also acknowledge the support by a grant from the Korea Basic Science Institute (Project No. 3372A). References [1] Nell R. McQuaker and Mary Gurney. 1977. Analytical Chemistry 49: 53-56 [2] E. Sanz, R. Munoz-Olivas, C. Camara. 2005. Analytica Chimica Acta 535: 227-235. *Acknowledgement* - This research was supported by grants from the Korea Basic Science Institute (Project No. C3372A and K33801).

WE113

A comparative study of rare earth elements (REEs) ecotoxicity. Implications on risk assessment.

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The Rare Earth Elements (REEs) are a group of metals, including lanthanides, ytrium and scandium, with a strategic importance in several economic sectors (agriculture, medicine, motor industry...). As a consequence, their global production has increased exponentially in the last decades and human use is disrupting their biochemical cycles (e.g. gadolinium anomalies in freswater and tap water, REEs enrichment of soil as consequence of agricultural practices). For lanthanides, we know that their atomic properties vary in a predictable way, but it is remain unclear if these elements show any coherent pattern for ecotoxicity, which may be useful to identify which prioritization measures are needed to manage these emerging contaminants. The French ARN project Labex Ressources 21 proposes an approach to the understanding, exploitation and environmental management of strategic metal resources for the 21th century. In this framework, the aquatic ecotoxicity of three lanthanides: Cerium (Ce) as a light-REE, Lutetium (Lu) as a heavy-REE and Gadolinium (Gd) with an intermediate position, was studied using a test battery composed by: crustaceans (*Daphnia magna* and *Heterocypris incongruens*), rotifer (*Brachionus calyciflorus*), hydra (*Hydra attenuata*), alga (*Pseudokirchneriella subcapitata*), bacteria (*Vibrio fisheri*) and photosynthetic enzyme complexes (PECs). The organisms were exposed to seven different concentrations (100-6400 μg/L) according to standard guidelines and metal concentrations were measured. Biological responses were modeled with a species sensitivity distribution (SSDs), and site specific anomalous REEs concentration were plotted against the SSD. Ecotoxicity of lanthanides seems to increase with increasing atomic number (Ce

WE114

Monitoring of soil pollution in the area of Kara-Balta

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During 50 years the consequences of mining companies and processing plants have being influenced on the environment of territories of Kyrgyz republic. From 1955 y the uranium processing plant had functioned till 2000 y. The plant was located in Kara-Balta (Kyrgyzstan). At 1.5 km from the city the uranium waste tail was formed. The mining processing plant in Kara-Balta constructed tail near the inhabitant localities. It was not well sealed. The radioactivity near this territory is 25-550 mcR/h. Besides that the pollution of environment with chemical components have being continuing. The special attention in ecological monitoring the soil is demanded. This is the most important component for biogeocenoses and biosphere at all. The soil accumulated heavy metals during long periods. Migration of them may be through alkility, plants growth and development and other processes. XRF spectrometry Delta method was used for research of soil pollution. The territory for research was 2- 4 ha near the tail and adjoining to farmer territories. The pollution with heavy metals had no uniform distribution according to radiation gradient and distance from the tailing. It showed that the accumulation of them in the soil depends on many factors such as industry, transport activity,

agriculture activity, migration of wastewaters. We detected the Cr concentration from 77 ±15 mg/kg till 508 ±42 mg/kg that 30 time higher than allowable. Cu – from 28±5 mg/kg till 515±27 mg/kg (trice higher). The concentration of Zn at the same territory was changed from 25 ±9 mg/kg till 2052± 50 mg/kg. It is also demonstrated increased content in the soil of detected area. Pb migrates usually in bicarbonate forms and being absorbed by clays. That's why in the areas where the soil is presented by clays the concentration of Pb is so high (836±25 mg/kg). But for control area the concentration of Pb was 25±5 mg/kg. The landscapes without anthropogenic activity usually demonstrate low concentration of heavy metals, (mg/kg) Cd in soil – 0.5, Hg – 0.01, Pb – 16, Cu – 100. Our results demonstrate the pollution of soil with many not only heavy but light metals. Their content in samples were also higher. Probably the presence of tail, transport and waste water increase the regional pollution in investigated area of Kara-Balta. For successful monitoring of environment we also need to receive data about background concentration heavy metals in our areas. Acknowledgements. This work is supported by the ISTC grant (project KR-2093).

WE115

Petroleum hydrocarbon contamination of urban soils in Accra, Ghana

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The use of petroleum products such as lubricating oils and fuels at auto-mechanic workshops in Accra, Ghana results in the contamination of soils because the workshops are mostly located on unsealed areas. Substances used in the workplaces are disseminated to the environment directly. For the purpose of retaining the soil and reducing dust formation large quantities of waste oil are spilled on the ground. Mineral oil-based oils and greases and their additives are classified as environmentally hazardous. Aged oils which are frequently used in Ghana may contain heavy metals or organic compounds such as plasticizers, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). The fate of contaminants remains unknown, an entry into water bodies by runoff can be assumed. However, studies conducted to determine the local loads are missing until now. Soils of five auto-mechanic workshops located in urban and suburban areas of Accra and of one diesel tank station in the neighbouring city Tema were investigated. Samples were collected randomly from each site within a depth of 0 to 15 and 15 to 30 cm. Control samples were taken from nearby grass verges. Extraction of petroleum hydrocarbons (TPH) was done according to a standardized procedure by extracting the sample with acetone and n-heptane. The detection was performed by FID after gas chromatography. Concentrations of PAHs, PCBs, heavy metals and rare-earth elements were examined. Standard parameters such as soil texture, carbonate, EC, pH and dry matter were additionally determined. Results show a partially very high contamination with TPHs with levels up to > 30 g kg^{-1}. A shift into deeper layers of soil increases with higher sand content and can be especially observed for short chained, aliphatic compounds. Methylated PAHs and di(2-ethylhexyl) phthalate (DEHP) were identified in heavily-loaded sites. Metal contents (mg kg^{-1}) in the soils ranges as follows: Fe (3000-134 000), Pb (2.7-8600), Cu (2.3-803), Ni (1.54-127), Cr (7-587), Cd (0-2.9) and Mn (27-1624). The highest concentrations were measured in soils in the centre of Accra. There are no correlations between TPH contamination and metal contents. Results provide first evidence of the loading situation. Large-scale investigations and research on water bodies and urban gardening products could provide more information about the extent of the contamination and help developing solutions to mitigate the loading situation.

WE116

Desorption and bioavailability of polychlorinated biphenyls, polycyclic aromatic hydrocarbons and heterocyclic compounds present in natural sediments

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Sediments as an important factor influencing water quality have become a central topic of the scientific and public discussion. While the quality of surface waters in Germany has considerably improved during the past decades, historically contaminated sediments still pose a significant risk to the quality goals of the European Water Framework Directive. A multitude of studies concerned with the evaluation of sites contaminated with polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and heterocyclic PAHs often only state total contaminant concentrations (as determined using exhaustive extraction techniques, such as Soxhlet or Pressurized Liquid Extraction). But for a comprehensive risk assessment and management, bioavailability and desorption kinetics of these compounds provide additional information, leading to more realistic scenarios. The present work was conducted within the dioRAMA project, a joint research framework between the Institute for Environmental Research of the RWTH Aachen University and the German Federal Institute of Hydrology (BfG). The aim was to determine both bioavailability and desorption characteristics of PCBs, PAHs and heterocyclic PAHs present in three different sediments from the German rivers Rhine (Ehrenbreitstein) and Elbe (Magdeburg, Prossen). While sediment from the harbor Ehrenbreitstein in Koblenz was chosen as representative for moderate contaminated sediments, sediments from the "Zollelbe" in Magdeburg and from Prossen (close to the Czech border) were chosen for reflecting highly contaminated sediments. For this, common roach (*Rutilus rutilus*) were exposed to the respective sediments for 28 days in a recirculating system. Sediments and water were exchanged every 10 days. Samples of sediments and fish were taken after 4, 7, 14, and 28 days exposure and extracted by means of pressurized liquid extraction. The extracts were then analyzed for PCBs, PAHs and heterocyclic PAHs by means of gas chromatography-mass spectrometry (GC-MS). Furthermore, the desorption rates of the pollutants were analyzed in a sediment/water system using the 'infinite sink' method TENAX as adsorbent.

WE117

A novel method for sediment extraction and cleanup for chemical and effect screening of polar contaminants

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Sediments are well known as a sink for 'classical' and highly hydrophobic persistent organic pollutants such as PAHs, PCBS or brominated flame retardants. Recent studies indicate that more polar or ionic compounds such as pharmaceuticals and personal care products may accumulate in sediments as well and contribute to a large extent to the observed toxicity, particularly if bioavailability is taken into consideration. To enable the targeted and untargeted chemical screening of polar contaminants as well as biotesting and effect-directed analysis (EDA) of sediments with a focus on polar contaminants, we set out to develop a new extraction and clean-up procedure. To this end, we used sediment samples spiked with a broad set of target compound from different classes covering a wide range of physico-chemical properties and functional groups. The method is based on a multi-step pressurized liquid extraction (Dionex ASE 200) and was optimized with regard to extraction temperature, solvent composition and number of extraction cycles for balanced recoveries of many compounds. Current studies focus on the optimization of a cleanup procedure to remove macromolecular compounds and elemental sulfur, which interfere with the biotesting and chemical analysis. For detection, we use liquid chromatography (LC) coupled to high resolution mass spectrometry using both electrospray and atmospheric pressure photo ionization, which enabled us to cover a wide range of compounds with complementary techniques.

WE118

Development of effect-directed analysis methods for amphiphilic substances in sediment and phytobenthic communities.

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The wide use of surfactants in our society (e.g., in personal care products, pharmaceuticals, detergents, and pesticides) makes their presence rather ubiquitous in the environment. Previous works suggested the ecotoxicological relevance of this group for the aquatic life. However, due to their specific surface-active properties and thus requirements in analysis and bioanalysis surfactants have been extensively ignored in effect-directed analysis so far. Challenges include the extraction of the whole group of surfactants together with a broad mixture of contaminants from sediments and biota, but also fractionation and biotesting controlling losses to surfaces and maintaining well defined exposure. This is further aggravated by the different behavior of anionic, cationic and non-ionic surfactants. Their amphiphilic property makes the use of attached living organisms relevant to assess their toxicity, as phytobenthos is one of the first communities which tend to cover stream beds who are in contact with these chemicals. As well the phytobenthic communities are an important chain of the food web. The present poster will provide new data on the development of extraction and analytical methods for a broad range of surfactants including e.g., QACs, betaine, PFC, and LAS from environmental matrix (i.e. sediment, biofilms and cladophora) and on the assessment of their impact on biofilm communities. Method development includes the adaptation of existing Accelerated Solvent Extraction (ASE), and Gel Permeation Chromatography (GPC) clean-up to the specific requirements of surfactants, biofilm-based assays with well-defined and controlled exposure and LC-ESI-MS/MS analysis. This preliminary work is a crucial step on the identification of toxic surfactants to aquatic biofilm by EDA, as it gives information

on the presence of typical amphiphilic substances in WWTP influenced stream for a further case study.

WE119

Identification of tetra propyl ammonium in river Rhine with LC-Orbitrap MS

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At the border of the Netherlands and Germany, where the river Rhine enters the Netherlands, the International measuring station monitors the water quality on a daily routine basis. This monitoring consists of specific target compounds but also non target compounds found with LC-UV, GC-MS or LC-MS. When a compound is found with a relative response higher than 3 ug/l, a warning is sent to selected water agencies, such as drinking water companies. The unknown compounds are commonly identified by using mass spectral libraries such as NIST and custom built accurate mass databases. When the compound is not found in the libraries, structural elucidation is necessary. With the accurate mass spectral information and fragmentation patterns obtained with the Orbitrap mass spectrometer, structure formulas can be identified. When it is not possible to build the structure from the formula, additionally techniques as NMR can be used. Here the identification of the occasionally found tetra propyl ammonium is presented.

WE120

ILLICIT DRUGS OF EMERGING CONCERN IN SURFACE AND WASTEWATER

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A monitoring program was undertaken in Valencia in Spain to determine the fate of emerging illicit drugs in three contrasting wastewater plants utilising different wastewater treatment technologies. The impact of treated wastewater effluent on the quality of receiving waters was also assessed. Samples were collected at 25 sites through the Turia River Basin and wastewater samples were collected from the influent of three wastewater treatment plants (WWTPs) in Valencia. The 8 emerging illicit drugs selected for this study were α- pyrrolidinopropiophenone, α-pyrrolidino-pentiophenone, 4'-methyl- α-pyrrolidinohexanophenone, 4'-methyl- α-pyrro-lidinobutiophenone, belong to pyrrolidinophenone group, Mephedrone, Dibutylone, 4-Methoxyphencyclidine and Bufotenine. Illicit drugs were extracted from 250 ml of water by solid phase extraction (SPE) and determined by liquid chromatography triple quadruple mass spectrometry (LC-QqQ-MS/MS) using an electrospray ionization source (ESI) in positive ionization mode. The method detection limits ranged from 0.01 to 1.54 ng L^{-1} and the recoveries from 57 to 127 % with relative standard deviations ≤ 20 % The feasibility of this method was demonstrated by analyzing spiked water samples. Their application to determine new illicit drugs in the influent of the selected WWTP shows the presence of bufotenine in all samples (at concentrations up to 325 ng L^{-1}) and 4'-methyl- α-pyrro-lidinobutiophenone in 24 % (at concentration up to 240 ng L^{-1}). Also, bufotenine was detected at 7 sampling points of the Turia River Basin at concentrations ranging from 4 to 67 ng L^{-1} and 4'-methyl- α-pyrro-lidinobutiophenone was detected only in one point at 38 ng L^{-1} for BUF. The others illicit drugs analyzed were not detected neither in the influent of WWTPs nor in Turia River Basin. To our knowledge, it is the first time that these drugs were detected and monitored in River Basins. This study also highlights the need of future research regarding these drug's transformation pathways and their ecotoxicological effects.

WE121

Development of an affordable and useful analysis for the measurement of pharmaceuticals in surface water

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Pharmaceuticals have been recognized as potential hazardous contaminants since their identification in water. However, there is no legislation about their emission to water. Moreover, none are yet considered as priority substance under the Water Framework Directive and there is no agreement on an EQS as yet. This might be due to the fact that the group of pharmaceuticals is divers in polarity, solubility and persistence. Hence, their behavior in water. This hampers effective analyses, and thus effective monitoring and pollution control measures. In 2012, Rijkswaterstaat collaborated in the Dutch ILOW[1] project that aimed at the development of an affordable and readily applicable analysis for the measurement of pharmaceuticals in surface water. Partners in the project are the Dutch foundation for Applied Water Research (STOWA), Grontmij and Dutch water boards. This method of analysis allows the measurement of a selective set of human pharmaceuticals that could be representative for a diverse range of these compounds (and their residues). In addition, it will provide a tool for monitoring the effectiveness of pollution control measures taken by policy makers. The main challenges in this project are the selection of a small set of representative pharmaceuticals, with a wide range of chemical and physical characteristics that can be measured within one method of analysis. The initial selection of pharmaceuticals is based on their occurrence in Dutch surface water, literature studies, and the appearance on watch lists. Subsequently, removal rates (WWTP), (eco) toxicological aspects and factors as commercial availability of an analytical standard were included. The final selection contained ± 28 pharmaceuticals from different groups (antibiotics, X-ray contrast agents, β blockers, analgesics), with different removal rates (WWTP) and with the highest possible environmental risk. Finally, a method was developed for the determination of the selected pharmaceuticals in surface waters. The method using offline solid phase extraction with LC-MSMS is validated according to NEN-EN-ISO/IEC 17025. Subsequently, the method will be applied for monitoring at several sampling locations in the main water systems of the Netherlands. [1]

ILOW: Integraal Laboratoriumoverleg Waterkwaliteitsbeheerders

WE122

Pharmaceuticals as indicators of wastewater pollution in marine sediments from an urbanized bay of Korea

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Concerns have emerged regarding the presence of human-use pharmaceuticals in aquatic environments. Because of relationship between pharmaceutical concentrations in the environment and human waste, recent studies have reported the presence of selected pharmaceuticals in wastewater effluent and river water. Few data exist regarding their presence in fresh sediments. To date, there is only little information about the occurrence and distribution of pharmaceuticals in marine sediments. To our knowledge, this is the first report on occurrences and distributions of pharmaceuticals in marine sediment of Korea. We investigated the status of contamination by 29 human-use pharmaceuticals as well as wastewater indicator compounds, fecal sterols and the synthetic endocrine disruptor nonylphenol, in marine sediments from the urbanized and semi-enclosed Masan Bay of Korea. Among the 29 pharmaceuticals determined, 10 including antacid, analgesic, antibiotic, and antipruritic compounds, and metabolites of caffeine and nicotine were detected in all sediment samples. Cimetidine, acetaminophen, and 1,7-dimetylxanthine were the most frequently detected pharmaceuticals, (frequency>50%) and at high concentrations. The highest concentrations and detection frequencies were at stations located close to wastewater treatment plant (WWTP) outfalls and at the river mouth. The spatial distributions of pharmaceutical were significantly correlated with those of wastewater compounds. These results indicate that occurrence of the pharmaceuticals in marine environments is likely associated with direct sewage inputs, such as WWTP effluents and with other sewage-influenced sources, such as river discharge.

WE123

Determination of an Indicator Micropollutant in Surface Water and Water Treatment Plant

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Micropollutants have been discharged to surface waters by the untreated effluents in sewage treatment plants (STPs) and wastewater treatment plants (WWTPs). The contaminated waters are naturally utilized as a source of drinking water in water treatment plants (WTPs). Most of the micropollutants resist conventional WTP systems and survive in tap water. In particular, pharmaceuticals and endocrine disruptors (ECDs) are examples of frequently detected micropollutants in drinking water. For the estimation for the effectiveness of micropollutant removal in the specific treatment process, indicator micropollutants can be selected through the monitoring of their fates and removal. In this study, in order to estimate the suitability as indicator micropollutants in water treatment plants (WTPs), caffeine (CFF), metoprolol (MPT), sulfamethoxazle (SMZ), and carbamazepine (CBM) were selected and were monitored in various waters such as river, spring, tap, and bottle waters with LC/MS/MS. Also, the lab-scale experiments of conventional treatments (coagulation, adsorption, and chlorination), and advanced oxidation processes (AOPs; ultraviolet (UV-C) radiation, UV/chlorine (Cl_2/UV), UV/peroxide (UV/H_2O_2), and ozonation (O_3)) were conducted to confirm the persistence of the selected micropollutants in WTPs. All selected indicator candidates were detected > 80% in river sample, and the accumulation of these micropollutants was observed in the downstream of river. Bottle water and spring water samples had no detection for these micropollutants. CFF showed the highest levels (158.6 ng/L) and positive correlation in the co-occurrences in river samples. However, the higher level of MTP (14.2–40.4 ng/L) was found in tap water than that of CFF. For treatment point of view, MTP showed the lowest removal (10–18%) in the conventional water treatments. Adsorption was effective to the removals of CFF, SMZ, and CBM (>70%) although they resisted to coagulation and chlorination. Among the applied AOPs, combining chlorine with UV reaction was the most effective for the removals of the selected micropollutant (90–100%). Considering the detection in various water samples and resistance in drinking water treatment system, MTP was recommended as an indicator micropollutant.

WE124

Screening and target analysis of emerging contaminants in waste and surface water in the river Danube in the surrounding of the point at the 1255 km

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The wastewater, municipal and industrial, is discharged directly into the Danube surface water at four locations in the vicinity of Novi Sad. The samples of waste and surface water were analyzed in three screening and two target campaigns. A 800 ml aliquot of water sample were spiked with internal standard (phenanthrene-D10 in case of PAHs and industrial chemicals and propazine or cis-chlordane in case of pesticides and mass labeled PBDE-138 -Wellington laboratories) to achieve final concentration of 1µg/l and extracted with two 50 ml portions of dichloromethane for 20 minutes. After extraction, both extracts were combined and dried with anhydrous sodium sulfate. Small aliquots of copper powder were added into obtained extract to remove elementary sulphur. After filtration, the combined extract was evaporated using Kuderna-Danish apparatus to final volume of 1 ml. A 50 µl of extract was injected into Agilent 6890 gas chromatograph with Agilent 5973 mass spectrometric detector. The GC system was equipped with PTV injector that was programmed from 60 ˚C to 260 ˚C (5 minutes) at a rate of 40 ˚C/min. Capillary GC analysis was performed on a 30 m x 250 mm I.D., 0.25 mm df DB-XLB and HP-5MS column. Helium was used as carrier gas. Simazine, Atrazine, Isoproturon, Diuron and hormones were analyzed using SPE-HPLC-DAD according to modified ISO 11369 procedure. Some of compounds such as diclofenac, galaxolide, simazine, atrazine, alachlor, bifenox, cybutryne, cypermetrine, methyl jasmonate, 1,2-benzisothiazole, 2-(methylthio)-benzothiazole and triphenyl phosphate were measured under LOD at all sampling sites in both target campaigns. The most frequently occurring contaminants in studied water samples were phthalates, phenols, PAHs, aromates and alkanes. Diethyl and dibutyl phthalates were identified in all studied samples indicating the pollution of surface and waste water from wild landfills, as well as from mixture of waste oils. This type of research was performed for the first time in the river Danube in the surrounding of the point at the 1255 km within NATO International Project. Acknowledgement: The research has been supported by Ministry of Education, Science and Technological Development, Republic of Serbia (III46009), Bilateral Project No. 680-00-140/2012-09/13, SRDA project No. SK-SRB-0022-11 and NATO Science for Peace Project „Drinking Water Quality Risk Assessment and Prevention in Novi Sad municipality, Serbia“ (ESP.EAP.SFP 984087).

WE125

ADAPTATION OF A METHOD FOR SIMULTANEOUS CHEMICAL ANALYSIS OF ANTIFOULING BIOCIDES

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The biocides used to paints, wich act as antifouling, being a groups of substances potentially adverse to aquatic ecosystems. Antifouling paints are treatments to minimize corrosive processes on ships and port's structures, reducing maintenance costs, saving fuel and reducing the transmission of non-native species of coastal ecosystems.The formulation of antifouling paints can include, inorganic, organic or organometallic substances. These prevent the growth and development of fouling organisms such as bacteria, macroalgae, mussels, and other invertebrates. The use of antifouling paints to ensure proper protection and reduces the mechanical strength of the vessel, reduces friction between the hull and seawater, thus increasing the operational efficiency of these structures. This study aimed to optimize the methodology for determination of antifouling biocides, chlorothalonil, Dichloflunid diuron, Irgarol and take samples of water and sediment. The biocides were analyzed using a liquid chromatograph Agilent ® 1200 series with diode array detection (G1315D). The analytical methodology was a short modification of the parameters described by Thomas et al (2002) e Sánchez-Rodríguez et al (2012). The elution program used was, a mobile phase consisting of methanol and ultapure water (A:B): 3 min: 50:50% (A:B)/ 14 min: 80:20% / 3 min to achieve initial equilibrium. The analitical curves were made at a concentration range between 0.5 to 5 mg L^{-1}. There is a linear relationship between the peak areas obtained and concentrations of analytes, with correlation coefficient ($r > 0.990$). In order to optimize the methodology the same separation conditions will be applied to an LCMS / MS, then the recovery study will be done using water and sediment, and application of the method on samples of port regions. Based on what has been observed, the proposed method is suitable for determination of antifouling biocides in different samples of marine and estuarine ecosystems of the Brazilian port area.The analytical method for the simultaneous determination of anti-fouling reinforcement in samples of marine sediments and water was optimized. These data are preliminary, but significant, considering knowing the trends of environmental pollution by antifouling compounds on marine and coastal ecosystems.The results demonstrate that the proposed method can be an effective tool for assessing the dynamics of antifouling biocides in estuarine and marine ecosystems.

WE126

Aquaculture area using expanded polystyrene buoys can be an area of concern for HBCD contamination in the marine environment

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Hexabromocyclododecanes (HBCDs) are the third most highly produced brominated flame retardant. Main applications of HBCDs are in polystyrene foams and textiles as additive flame retardants. For the last decade HBCDs were widely investigated in the environment due to their potential persistence, bioaccumulation and toxicity. Due to these properties, HBCDs are currently designated as one of persistent organic pollutants in Stockholm Convention. In this study, the contamination characteristics and pollution level of HBCDs are investigated in a semi-enclosed bay of South Korea. The HBCD concentrations in sediment were in the range of 0.78 to 49.92 ng/g dry weight. Spatial distribution of HBCD was not consistent of those of other persistent organic pollutants, implying different source influence. Although most of persistent organic pollutants such as PBDEs, PCBs, organochlorine pesticides and PAHs showed high concentration in the inner part of the bay where big cities, industrial complexes, and a sewage treatment plant were located, HBCD showed the highest concentration near the oyster farms located in the outer part of the bay. At the follow-up survey on aquaculture farm area, a much higher concentration of HBCDs was detected in its bottom sediment. Recently, we found expanded polystyrene (EPS) buoy contained HBCD as an additive. HBCDs can leach out from EPS buoy to the water column, and finally be accumulated nearby bottom sediment. This is the first observation that aquaculture area using EPS buoy can be an area of concern for HBCD contamination in the marine environment. In addition, a negative concentration gradient of HBCDs was observed with a distance from the outfall of sewage treatment plant (STP), indicating STP is another source of HBCDs in this bay.

WE127

Occurrence of organophosphate flame retardants and plasticizers in seawater from the South China Sea

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Flame retardants (FRs) are the chemicals used for increasing fire resistance of materials. Since the worldwide restriction on the production and use of polybrominated diphenyl ethers (PBDEs), the use of other non-regulated flame retardants such as organophosphate flame retardants (OP-FRs) have been increased to meet flammability standards. Over the past several decades, coastal pollution has become a great concern in South China due to rapid industrialization and urbanization. Therefore, it is believed that various forms of flame retardants such as OP-FRs widely occur in this region. However, little is known about their occurrence and fate in this coastal environment. The main objective of this study is to investigate the current status and distribution of non-halogenated flame retardants namely OP-FRs and plasticizers in the South China Sea by analyzing the levels of thirteen OP-FRs in the seawater. The OP-FRs in this study included tris(2-butoxyethyl) phosphate (TBEP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), triphenyl phosphate (TPhP), tri-*iso*-butyl phosphate (TiBP), tri-*n*-butyl phosphate (TnBP), tripropyl phosphate (TPrP), triphenyl phosphine oxide (TPPO), triethyl phosphate (TEP), trimethyl phosphate (TMP), tri-*iso*-propyl phosphate (TiPrP) and tris(1,3-dichloro-2-propyl) phosphate (TDBPP). These compounds were identified and quantified by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method and the procedural recoveries of all OP-FRs ranged from 77% to 117%. The spatial differences and the possible sources of OP-FRs in the South China Sea will be discussed in this paper.

WE128

Identification strategies for halogenated flame retardants and related chemicals

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Recently, there has been an increase in the use of accurate mass instruments (e.g. TOF-MS) and spectra-less databases (based on mono-isotopic mass alone) for the identification of “unknowns”. Since the phase-out of the penta-brominated

diphenyl ether (Penta-BDE) and Octa-BDE mixtures, other chemicals have started to be used as flame retardant replacements. The instruments used in the present study are a Bruker Daltonik microTOF II-MS (mass accuracy < 2 ppm and resolution >16500 FWHM), with an APCI source, coupled either with an Agilent 7890A GC or an Agilent 1220 LC system, and an Agilent 1290 LC coupled to an Agilent 6530 QToF-MS, with an ESI Jetstream source. \n Dust samples from previous studies such as house dust from California (n=5) and dust from e-waste storage areas in Thailand (n=6), along with samples of car interiors (n=8) and consumer products from The Netherlands (n=4) were screened for other chemicals than those targeted in previous analyses. The LC-TOF-MS chromatograms were processed employing the “Dissect peak” facility from the Bruker Data Analysis software, the “Find by Molecular Feature” facility from the Agilent MassHunter Qualitative Analysis and visual inspection, screening for halogen clusters of 4 or more atoms. For the fragments of interest, elemental formulas were generated using such as the Bruker Smart Formula or Agilent Generate Formulas. Using this approach, many highly halogenated, high mass “unknowns” were detected. By doing searches in spectra-less databases (e.g. the ChemSpider service, with >28 million entries) and by process of elimination, two of the hexachlorinated analytes (one in positive, the other in negative mode) were identified as V6, a recently detected FR found in 2 US dust samples (structure confirmed by MS/MS experiments and by comparison with the reference standard) and ethyl 4-[4,6-bis(trichloromethyl)-1,3,5-triazin-2-yl]benzoate (found in dust samples – 4 from the US, 5 from Thailand and in one of the car interior samples). This latter chemical is used as initiator in the production process of a number of polymeric materials.

WE129

Atmospheric deposition of polybromodiphenyl ethers in remote mountain regions of Europe

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Polybromodiphenyl ethers (PBDEs) were analyzed in bulk atmospheric deposition collected in four European remote mountain areas over a period of two years (2004-2006): Lake Redon (Pyrenees), Gossenköllesee (Alps), Lochnagar (Grampian Mountains) and Skalnate (Tatras). In all sites, the PBDE distributions were dominated by BDE209. BDE47 and BDE99 were the major low-brominated congeners, followed by BDE100 and BDE183. This composition is consistent with predominant inputs from the commercial mixtures decaBDE and pentaBDE. The total congener site-averaged fluxes ranged between 100 ng m^{-2} mo^{-1} (Alps) and 190 ng m^{-2} mo^{-1} (Tatras). Significant correlations between PBDE deposition and percent of North Atlantic backwards air mass trajectories in the collected samples of the westernmost sites, Lochnagar and Redon, suggested an impact of transcontinental transfer of these pollutants from North American sources into Europe. Skalnate and, to a lower extent Redon, recorded another main PBDE source from Central Europe corresponding to secondary emissions of the penta-BDE commercial mixture. The fluxes of these secondary emissions were temperature dependent and correlated to total particle deposition and rainfall. Higher PBDE fluxes were observed at increasing temperature, particle deposition and precipitation. Another specific PBDE source was observed in United Kingdom and recorded in Lochnagar. Photolytic degradation during transport decreased the relative abundance of BDE209 and modified the emitted pentaBDE technical mixtures by depletion of the relative composition of BDE99 and, to a lower extent, BDE47. The transformations were more intense in the sites located above 2000 m, Redon and Gossenköllesee, and, particularly, during the warm periods.

WE130

Alternative halogenated and organophosphate flame retardants: estimated physical-chemical properties and persistence in indoor and outdoor environments

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In the wake of the listing by the Stockholm Convention of HBCD, pentaBDE, and octaBDE and the nomination of decaBDE, an increasing number of alternative halogenated flame retardants (FRs) are being used in products. Some replacement FRs are being found in indoor dust, urban watersheds and remote Arctic air. Though currently in use, there is very little information regarding the physical-chemical properties, environmental fate, and persistence of these chemicals. As such, we compiled a list of over 70 compounds that are used or marketed as halogenated flame retardants (HFRs), including 58 that are brominated, 10 chlorinated, 5 containing Br and Cl, and 7 halogenated organophosphate compounds. The physical-chemical properties of the FRs were obtained using the QSAR models EPIsuite, SPARC and Absolv. Values of $\log K_{OW}$ ranged from -0.52 for 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ) to 16.49 for bis(pentabromobenzyl) terabromophthalate (BPBTB). Values of $\log K_{AW}$ ranged from -18.83 for N-N-Ethylene-bis(tetrabromophthalimide (EBTEBPI) to -0.96 for Mirex. Values of $\log K_{OA}$ ranged from 4.84 for dibromoethenyl-benzene (DBS) to 30.20 for BPBTB. Variation and uncertainty in the estimates increased for compounds with polar functional groups and heteroatoms in the structural backbone. Their overall persistence (P_{ov}) and long-range transport potential (LRTP) were modelled using the OECD P_{ov} & LRTP Screening Tool. 58% of the HFRs had a characteristic travel distance (CTD) of over 2000 km, or a high LRTP, while 12% had a CTD of 700 km to 2000 km and 30% of 700 km or less. The P_{OV} increased with molecular weight up to 450 g/mol, beyond which P_{OV} was estimated to be approximate 8 years in the outdoor environment. Persistence indoors was modelled using an adapted Multi-media Indoor Model (MIM). For comparative purposes, HBCD, tetra-, penta-, octa-, and decaBDE were also modelled.

WE131

Assessment of legacy and emerging persistent organic pollutants (POPs) in southern hemisphere Humpback whale blubber: A non-target screening approach

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Southern hemisphere Humpback whales (Megaptera novaeangliae)feed almost exclusively on Antarctic krill. The lipophilic nature of many POPs combined with their long-half lives, makes whale blubber a particularly interesting matrix for studying bioaccumulation and bio-magnification of both natural and anthropogenic pollutants. The high fidelity diet of southern hemisphere Humpback whales enables us to gain precious information about the state of the contamination of the Antarctic food web through the analysis of blubber biopsies of free-ranging individuals. This study aims to apply a GC/MS non-target screening technique for further expanding and evaluating patterns of historical and emerging compounds. Circa 30 biopsies were collected through a minimally-invasive biopsy method from free-swimming individuals during their late migration journey in September/October 2013 along the coast of North Stradbroke Island, Queensland, Australia. Samples will firstly be analyzed by gas chromatography (GCXGC-MS), for the determination of the major classes of persistent organic pollutants: current and historic-use organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). This study builds upon previous team work which has monitored OCPs and PCBs in the target population of whales since 2007 and the values we will contribute toward temporal trend data. Moreover Time of Flight(ToF-MS) full spectra acquisition will allow us to register more environmental contaminants without a pre-selection of the structures of interest: we will collect all signals of all the potential compounds with chemical physical properties related to our target POPs. We expect this study to provide insight into the existing gap between the concentration of the pollutants we regularly detect, and the realistic concentration of molecules with toxic potential.

WE132

Occurrence and fate of different classes of endocrine disrupting chemicals in sewage treatment system and evaluation of their removal efficiency

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Endocrine disrupting chemicals (EDCs) are contaminants that can interfere with endocrine function in organism at low concentrations and are emerging as a major concern for water quality. Among various groups of EDCs, steroid hormones, alkylphenol ethoxylates (APEOs) and phthalate acid esters (PAEs) acquired high research attention and have been added into the priority pollutants list in EU and US due to their extensive application and estrogenic activity. Municipal sewage is one of the main routes by which EDCs are conveyed into the aquatic environment, and therefore it is important to increase our understanding of the EDC removal capacities of various wastewater treatment systems. To this end, the main objective of this work is to study the fate and behavior of the 33 target EDCs in different sewage treatment methods including chemically enhanced primary treatment (CEPT), primary sedimentation, activated sludge, disinfection process (UV, chlorination) and reverse osmosis (RO), and investigate their capabilities for eliminating these compounds by means of analyzing their levels in sewage, sludge and particulate samples using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method. In addition, the distribution of these compounds between dissolved and particulate phases was determined in order to understand their behavior in different sewage treatment methods. Of the 33 EDCs, levels of estriol, nonylphenol-mono-ethoxylate (NP1EO) and bis (2-Ethylhexyl) Phthalate (DEHP) were dominant in the influent samples with the concentration ranging from below limit of quantificaton-67.8, 722-8380 and 718-5120 ng/L, respectively. Concentrations of NP1EO and DEHP were also the most prevalent compounds in sludge and particulate samples, accounting for more than 60% and 99% of the total APEOS and PAEs resepctively. The removal efficiencies of primary sedimentation were relative lower (< 40%) for most chemicals. Activated sludge method was capable to eliminate more than two-thirds compounds with removal efficiency greater than 80%. The most efficient treatment method was the

advanced RO, which was able to remove almost all of these pollutants. The distribution coefficients between the absorbed and dissolved phase of APEOs and PAEs were positively correlated with the log K_{ow} for most of the treatment processes. Overall, the results demonstrated that the conventional wastewater treatment methods were not effective in removing EDCs.

WE133

Onsite large volume solid phase extraction – how to get 1000 litres of water into the laboratory?

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In recent years, research interests regarding the assessment of organic contaminants and their putative ecotoxic effects in surface waters river or lake systems shifted from hydrophobic to more hydrophilic organic compounds. Although modern LC-MS/MS techniques allow for the detection of these compounds at very low levels without pre-concentration, biological test systems still demand high concentration factors as achieved by solid-phase extraction techniques. For a successful application of chemical multi-target screening in combination with bio-test batteries or effect directed analysis including tests on higher trophic levels such as crustaceans, algae and fish the collection of hundreds to thousand litres of water is required. The appropriate storage and transport of such an amount of water is a not only a logistical hurdle, but also the timely filtration and SPE to avoid contaminant degradation. To overcome these challenges we developed an automated device for a large volume solid phase extraction directly at the sampling site. We demonstrate the successful field application of the device during the Joint Danube Survey 3 in summer 2013 using three sorbents based on a polystyrene-divinylbenzene copolymer in a row (CHROMABOND® HR-X to trap neutral compounds, followed by CHROMABOND® HR-XAW weak mixed-mode anion exchanger and CHROMABOND® HR-XCW weak mixed-mode cation exchanger) The experiences and results of this field test showed that it is feasible to extract 1000 litres of water onsite in 30 hours.

WE134

Technical Report on Aquatic Effect-Based Monitoring Tools in the context of the European Union (EU) Water Framework Directive

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In the mandate for 2010-2012 of the sub-group CMEP (Chemical Monitoring and Emerging Pollutants) under the CIS (Common Implementation Strategy) for the WFD (Water Framework Directive), a specific activity was foreseen for the elaboration of a technical report on aquatic effect-based tools. The activity was chaired by Sweden and co-chaired by Italy and progressively involved several Member States and stakeholders in an EU-wide drafting group. The aim of the report, which is expected to be approved in December 2013 by the Water Directors of the EU Member States, is to identify potential effect-based tools (e.g. biomarkers, bioassays *in vivo* and *in vitro*) that could be used in the context of the different monitoring programmes (surveillance, operational and investigative) under the WFD. The main objectives of using effect-based monitoring tools within the current WFD context would be: as screening tools, as part of the assessment of chemical pressures; to establish early warning systems; to take the effects of mixtures of pollutants or not-analysed chemicals into account (e.g. to support investigative monitoring where causes of a decline in certain species are unknown). Moreover the application of effect-based tools could result in a better understanding of the link between the chemical and ecological status of surface waters and provide additional support in water and sediment quality assessment. The report also contains specific sections on the use of such tools in marine contexts such as the Regional Seas Conventions and the Marine Strategy Framework Directive (MSFD). It includes a dedicated section on the use of effect-based tools in the different Member States and an annex that contains 14 case studies and a list of fact-sheets about the different tools. It also includes descriptions of tools and methodologies that are considered promising in the near future (e.g. effect-directed analysis, omics). This technical report, elaborated through close collaboration between the scientific community and regulators, can already be considered to provide important support to the managers, the assessors and the local operators involved in the analysis and monitoring of surface water, even though effect-based tools may not currently contribute directly to monitoring compliance with WFD quality standards. Based on the technical report, an applied project aimed at developing bioanalytical effect-based tools to support aspects of a new EU watch-list monitoring programme is in preparation.

WE135

Application of bioanalytical tools indicative of adaptive stress response pathways for water quality assessment

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The aquatic environment contains countless micropollutants and their transformation products, often at low concentrations, and routine chemical analysis is only able to detect a small fraction. Bioanalytical tools can be applied complementary to chemical analysis as they can detect all compounds that act by a certain mode of action, thus bioanalytical tools can assist with the identification and priorisation of emering micropollutants. For bioanalytical tools to be used for water quality monitoring it is important to include sensitive bioassays that act as early warning indicators of chemical exposure. Adaptive stress response pathways are only induced by chemicals or other external stressors, which trigger the activation of transcription factors that in turn initiate the transcription of cytoprotective genes and, thus, the expression cytoprotective proteins, with the goal to mitigate any detrimental effects induced and to restore the cell to homeostasis. Adaptive stress response pathways are also interesting for chemical monitoring as they occur ubiquitously in all cell types and are not restricted to specific tissue or organs. In this study, cell-based bioassays indicative of induction of the Nrf-2 mediated oxidative stress pathway (Nrf-2), p53 mediated apoptosis in response to DNA damage (p53) and inflammation (NF-κB) were applied to a range of water samples including wastewater effluent, stormwater and drinking water. With the exception of advanced treated wastewater, all samples induced oxidative stress, with the most response in the secondary treated wastewater effluent. The assays indicative of genotoxicity and inflammation were less sensitive, with only the most polluted samples giving a response in these assays. This study shows the potential of applying bioassays indicative of adaptive stress response pathways for water quality monitoring.

WE136

Chemical and bioanalytical characterisation of AhR agonists in PAH-contaminated soils during full-scale bioremediation

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Even though it is well-known that PAHs occur in the environment as complex mixtures of hundreds of related compounds, like oxygenated PAHs (oxy-PAHs), azaarenes among others, risk assessment and classification of PAH-contaminated soils are still based on a small number of PAHs, commonly the 16 priority PAHs. In the present study we used a chemical and bioanalytical approach to characterise PAH-contaminated soils before and after bioremediation. The objectives were to (1) examine the changes in concentrations of 43 PAHs, oxy-PAHs and azaarenes during the remediation process by use of GC/MS analysis, (2) use the H4IIE-luc bioassay, based on rat hepatoma cells, to evaluate if the aryl hydrocarbon receptor (AhR)-mediated toxic potential in the soils was reduced in proportion to the reduction in concentrations of the analysed compounds, by use of mass balance analysis, (3) measure the AhR-mediated activity in five arable soils and five soils sampled in different city parks, to use as safe levels for baseline toxicity in soils, and (4) estimate the amount of persistent compounds in the soils by use of both 24- and 72 h of exposure in the H4IIE-luc bioassay. Soil samples were collected from four biological treatment plants during time period 2010/2012, under on-going remediation processes. H4IIE-luc bioassay specific relative potency (REP) factors for 38 PAHs, oxy-PAHs and azaarenes were used in mass-balance analysis of the soils, to assess the contribution of chemically quantified compounds (chem-TEQ) to the overall AhR-mediated activity observed in the H4IIE-luc bioassay (bio-TEQ). In three of the soils, chemical concentrations and AhR agonistic activities were almost constant during remediation. Greater bio-TEQs than chem-TEQs were shown in all soil samples. The proportion of unknown AhR agonists differed between the soils. Although 43 compounds were included in the mass-balance calculations, only 2 to 20 % of the high AhR-mediated activities in the samples could be explained by the compounds analysed. Results from the 72 h exposure indicated that 10 to 30% of the AhR agonistic activity was from more persistent compounds in the soils. These findings show that traditional methodology of using chemical analysis of the priority PAHs to determine the degree of PAH contamination in soils greatly overlooks toxicologically relevant PAHs or other AhR agonists still present in soils after remediation.

WE137

Integrated chemical and toxicological methods for early detection of hazardous chemicals in drinking water

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The quality of tap water is considered to be high in Scandinavia. Recently, however, several water sources had to be restricted or even shut down because of chemical pollution from anthropogenic chemicals. In contrast to risks with pathogens, the occurrence of the hazardous chemicals was found by chance, and the drinking water treatment plants (DWTPs) were not aware of the risks.Thus, there is a need to identify presently unknown chemicals and to improve detection of known hazardous chemicals of anthropogenic and natural origin in drinking water to ensure future delivery of safe drinking water.The main objective of this research project is to develop methods for assessment of hazardous chemicals in drinking water by integrating chemical analysis and toxicity tests. Passive samplers are used to pre-concentrate hazardous chemicals in samples from source to tap. Sampling extracts are characterized in terms of chemical composition by advanced mass-spectrometry and toxicity by a battery of *in vitro* assays. The assays will measure cytotoxicity, endocrine disrupting effects, genotoxicity, oxidative stress and developmental toxicity in the concentrated water samples. Biobanked blood and urine samples are used for assessing the human exposure of identified hazardous chemicals. Different techniques for drinking water treatment are compared concerning effects on hazardous chemicals and toxicity, and the impact of dissolved organic carbon will be studied. The research will help stakeholders to improve monitoring strategies and to make well-founded decisions concerning investments in treatment technology for improved safety of drinking water, and for improved risk communication.

WE138

Estrogenic activities in water and sediment from French rivers using zebrafish-based bioassays

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Over the last decade, assessment of exposure of fish to aquatic endocrine disrupting chemicals (EDCs) and their effects on endocrine functions has become a major issue in ecotoxicology. In the present study, we explore the potential use of newly developed zebrafish (zf)-based *in vitro* and *in vivo* bioassays to detect estrogenic compounds in aquatic environment. For this purpose we use stable zf-estrogen receptor (zfER) subtypes (zfERα, zfERβ1, zfERβ2) in the zebrafish liver (ZFL) cell line and transgenic cyp19a1b-GFP zf embryos to screen organic extracts of sediment and water (using polar organic compounds integrative samplers - POCIS) sampled from 19 French river sites. Results showed no detection of estrogenic activities in sediment extracts by zf-based *in vitro* bioassays. Unlike zfERs *in vitro* bioassays, human receptor (hERα) *in vitro* bioassay was able to detect estrogenic activities in sediment extracts. However, POCIS-based bio-monitoring provide much more significant information on zf-based *in vitro* bioassays as estrogenic activities were mainly detected for most of the river sites. High numbers of POCIS extracts were found to be more active on zfERβ2 than on zfERα. The responsiveness of zfERβ2 to environmental samples is important as this estrogen receptor subtype is present in fish species but not in humans. In addition, SPE-based fractionation of POCIS extracts allowed distinguishing fractions that were active on zfERβ2 from those active on hERα. These results suggest a significant inter-assay difference (human MCF-7 versus zebrafish ZFL cells). Moreover, we found similar estrogenic activities on *in vivo* bioassay by sediment and POCIS extracts as observed on *in vitro* bioassays. Complementarily, this *in vivo* assay will allow taking into account the bioavailability and pharmacodynamics of estrogen mimicking compounds to enhance the efficiency and the accuracy of EDCs testing strategies. In summary, this study reports for the first time the use of fish-based bioassays, supporting the significance of using both *in vitro* and *in vivo* fish models to detect species-specific active contaminants in aquatic environment.

WE139

Miniaturization of the micronucleus assay to 96-well plate setup presents advantages for application in EDA

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The micronucleus assay is an important component of bioassay batteries that aim to assess the genotoxic and mutagenic potencies of chemicals and environmental samples. Micronuclei are formed when acentric chromosome fragments or whole chromosomes are unable to migrate to the poles during the anaphase stage of cell division. Resulting chromosome mutations are implicated in many human diseases, and in addition present risks to the environment. Current guidelines for the assessment of micronucleus induction, as the ISO 21427-2 for V79 Chinese hamster cells, recommend exposure setup in 6-well plates using cells cultivated on microscopic glass slides. This labor-intensive procedure reduces the test performance and requires relatively high amount of sample. In consequence, the bioassay application is restricted in analysis of samples with reduced volume, as for instance in Effect Directed Analysis (EDA). In our group, the micronucleus assay was optimized to 96-well plate exposure setup, by optimizing conditions for exposure, growth, and fixation of cells. Results from the exposure to model compounds, as 4-Nitroquinoline 1-oxide and Cyclophosphamide, were compared to results obtained in the 6-well plate micronucleus assay regarding the sensitivity of both setups. In addition, literature data for *in vivo* micronucleus in rainbow trout is considered for the discussion. The micronucleus in 96-well plate showed to be an efficient format for the assay, performing well for the detection of known mutagenic compounds. As an outcome, more samples can be tested simultaneously, reducing the test time and costs. Furthermore, the application of small sample amounts provides the evaluation of the full concentration response curve in case of positive samples. Thus, by incorporating the method to automated microscopic analysis, the improved micronucleus assay in 96-well plates is an accurate and cost-effective method, which significantly increases the throughput compared to the current guidelines.

WE140

The value of cell based and zebrafish bioassays for the evaluation of mutagenic compounds in EDA

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Mutagenicity assessment is a very important step in the evaluation of the toxic potential of chemicals and environmental samples. Recently, Effect Directed Analysis (EDA) has also identified mutagenicity bioassays as important tools for the identification of unknown environmental mutagens. Mutagenic compounds occurring in drinking water and aquatic systems represent a hazard for humans and for the organisms that inhabit these environments. Since mutagenicity presents threats to different biological levels (cell, organism, population, ecosystem), effects might develop into diseases in the exposed individuals, and in addition can reach the population level and future generations if gonads or early life stages are affected. Currently, different bioassays are available to measure and assess mutagenic effects. Among established methods, the micronucleus assay is considered to be a robust method that has been previously applied in *in vitro* and *in vivo* systems. However it is a work intensive and time consuming method, which reduces its applicability in EDA. On the other hand, the p53 CALUX assay is a recent promising tool, presenting advantages of similar cell-based assays that have great potential for high-throughput screening, and in addition it can provide additional information of metabolic activation or deactivation through incubation with S9-mix. Aiming to evaluate the value of these bioassays for mutagenicity assessment in EDA, the present work investigated the application of the p53-Calux and the micronucleus assay with zebrafish cell line and zebrafish early life stages. For the experiments, known mutagenic emerging pollutants with different mechanism of action were selected, as vinblastine (chemotherapeutic, aneugenic mutagen) and sodium arsenite (wood preservative and pesticide, clastogenic and aneugenic mutagen); plus one known not mutagenic compound (manitol); and the positive controls for the micronucleus assay and p53-CALUX (4-nitroquinoline 1-oxide and actinomycin D respectively). Results from the different assays are compared for sensitivity and specificity, and the potential for further application in EDA is discussed. As an outcome, this work contributes to the development of a mutagenicity testing strategy for the assessment of surface water samples and fractions in EDA. This project is funded by the Marie Curie ITN EDA-EMERGE.

WE141

planar-YES: steps towards a fast, robust and sensitive tool for effect-directed analysis of estrogenic activity

A. Schönborn, A.A. Grimmer, ZHAW

Effect-directed analysis (EDA) with bioassays (e.g., the yeast estrogen screen YES), is usually focussed on the detection of (toxicological) effects in complete samples. The results mostly don't allow drawing conclusions about the causes of an observed effect. This weakens the information value of the results and is one main reason why bioassays are not commonly considered as criteria for the assessment of water or wastewater quality. Using the cells of Routledge & Sumpter [1] as test

organisms and different estrogens as standards, we are developing a new YES variation (planar-YES) coupling thin-layer chromatography (TLC) with the yeast estrogen screen. In the detection step, sensitive cells are exposed to the sample by overlaying a TLC plate with the YES culture. The planar-YES procedure allows performing on one single TLC plate all steps including sample preparation, sample separation and the detection of bio-effective substances. It also offers a potential link to high-end chemical analytics. The basic functionality of this concept has been documented ([2],[3]). In our recent work, the planar-YES has been applied to waters of different provenience (treated wastewater, water from river bank filtration, river water, mineral water). We also developed a new procedure that allows omitting the laborious and time-consuming step of extracting water samples (e.g., by solid phase extraction). We demonstrated that a native water sample can be applied directly to a TLC plate by using a simple hand pipette, cleaned-up and developed subsequently. The sensitivity of the planar-YES and the range of volumes that can be applied to the TLC-plate allow to detect 17-beta-estradiol in concentrations lower than 1 ng/l, which is close to the AA-EQS proposed by Oekotoxzentrum [4] for this estrogen. The next steps of our research will focus on the direct detection of estrogenic substances in TLC-bands by using TOF-MS. [1] E.J. Routledge, J.P. Sumpter, Environ. Toxicol. Chem. 15 (1996), 241–248. [2] A. Schönborn, A. A. Grimmer, Journal of Planar Chromatography 26 (2013) 5, 402–408 [3] S. Buchinger, D. Spira, Journal of Planar Chromatography 26 (2013) 5, 395-401 [4] Schweizerisches Zentrum für angewandte Ökotoxikologie ETH/EPFL, Vorschläge für akute und chronische Qualitätskriterien für ausgewählte schweizrelevante Substanzen, Dübendorf (no date). Retrieved 01/22/2013, from http://www.oekotoxzentrum.ch/

WE142

Comparison of two simplified fractionation protocols for the assessment of organic contaminants in aqueous samples using effect-directed analysis

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Wastewater and surface water samples contain extremely complex mixtures of thousands of individual contaminants having different chemical properties and ecotoxicological characteristics. The assessment of the chemical status of a water body is therefore a challenging goal, which cannot be achieved by routine monitoring studies focusing on a limited number of target priority contaminants. As an answer to this inherent limitation, a more comprehensive approach, combining the effect assessment with a broad-spectrum chemical screening, has been proposed to address the problem of water quality and pollutant prioritization. The full-scale effect-directed analysis (EDA) however is a very demanding procedure since it involves extensive fractionation of the extracts, leading often to a large number of sub-fractions, which need to be analyzed by sophisticated chemical methods. Consequently, this complex and laborious approach is not suitable for a systematic application in large monitoring studies. Some recent studies suggested that significant progress in the assessment of priority pollutants could be achieved by introducing simplified EDA protocols, which comprise effect characterization and chemical screening based on a smaller number of well-defined fractions. The aim of this paper is the comparison two simplified fractionation protocols for the assessment of organic contaminants in aqueous samples. The comparison is based on the study of a wide range of organic contaminants, including classic contaminants such as polycyclic aromatic hydrocarbons and pesticides as well as several classes of polar emerging contaminants (e.g. pharmaceuticals, surfactants and their transformation products). A special emphasis was on the assessment of recoveries of individual contaminants, which is essential for the reliable interpretation of the effects observed in the original sample.

WE143

Evaluating hazardous pollutants from fire event: an EDA approach.

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Hundreds of chemicals are typically detected in environmental air samples from fire events and only a very small amount of these substances can be identified and then monitored. Furthermore, even if the plain pattern of chemical's species could be detected, often it does not explain measurable effects on biological systems, because a wide spectrum of toxicological outputs may derive depending on both exposure way (inhalation, but also dermal and systemic uptake) and chemical/physical parameters (*i.e.* chemical residue, relative concentration, burning trigger, environmental/fire temperature, *etc*.). In a fire event this context determines a site-specific toxicity, characterized however by large additive/ synergistic/antagonistic toxicological interactions, hazardous to ecosystems and human health. Understanding toxicological effects of a fire are much less advanced than other dangerous situations, obviously due also to the fleeting character of a fire, with very high difficulties in monitoring and sampling phases, as well as making the more appropriate analysis being the *a priori* unknown typology of the mixture originated from the burned substances. In fact, no world-wide recognized standard methods are nowadays available for evaluating the toxicity of combustion/pyrolysis products. Then, we used an EDA (*effect-direct analysis*) approach in order to evaluate the toxicity resulting from a mixture of chemical products springing from a fire. By a bio-diagnostic tool, *i.e.* a *in vitro* test called *FM22 bioassay* (Iero and Manente, *et al*., 2003), we performed an industrial mixture's burning, followed to fractionation and chemical structure elucidation by GC-MS. On respect of the chosen bioassay, being mitochondria ubiquitous in every kind of organisms (in all ecosystems), the mitochondrial respiration inhibition was the bioassay end point. EC50 has been determined for a lot of industrial substances (both products and sub-products) and matched with TEEL (*Temporary Emergency Exposure Limit*) values applied on GC-MS chromatogram analysis results.

WE144

Towards higher throughput in Effect-Directed Analysis

M. Lamoree,

In the past decades, Effect-Directed Analysis (EDA) – in which chemical analytical techniques are combined with (in vitro) bioassays to identify environmental contaminants capable of causing adverse effects – has developed into a promising tool for investigative monitoring. Research in this field was rekindled in the late nineties and focused on the identification of compounds that caused estrogenic effects in fish. By the implementation of assays covering other (endocrine disruption) endpoints than estrogenicity, such as (anti-)androgenicity and thyroid hormone disruption, the scope of EDA was widened. In addition, technological innovations in the field of analytical chemistry have contributed significantly to the potential to find, identify and quantify unknown compounds present in the environment. The application of accurate mass spectrometric techniques (time-of-flight, Orbitrap) in environmental analysis has been shown to facilitate the identification of unknown toxicants to some extent, but no major breakthrough in the identification of (known) unknowns has been achieved. In this poster presentation, examples of successful EDA studies will be given revealing the identity of (emerging) pollutants in sediments, surface waters and polar bear plasma. Despite these modest successes, the application and acceptance of EDA as a viable approach for the identification of compounds causing adverse effects has been delayed due to the laborious and time-consuming nature of the EDA work. When problems related to the relatively low throughput can be addressed, EDA may find its application in various fields, such as investigative water/sediment quality monitoring to support the corresponding policies, process control for e.g. biomass raw material treatment for biobased synthesis of commodity chemicals, for human and environmental exposomics, etc.

WE145

Optimization of an on-line solid phase extraction (SPE) coupled with UHPLC-MS/MS, for the determination of hormonal compounds in sewage from wastewater treatment plants of Gran Canaria (Spain)

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The aquatic environment and, consequently, the human health have been affected by organic pollutants since the last centuries and these adverse effects have been more evident in last decades. Steroid hormones are an important group inside of the emerging pollutants, and are considered as endocrine disruptor compounds (EDCs) due to their capacity of altering the natural hormonal equilibrium, producing harmful effects in animals, humans and their progeny. These changes are more noticeable in the marine environment [1]. The consumption of steroid hormones has increased exponentially in last decades, due to their use in human and veterinary medicine and the principal source of these pollutants are the wastewater treatment plants. Several studies have determined the presence of this type of compounds in wastewater samples [2]. Furthermore, steroid hormones do not need to stay a long time in the aquatic environment to produce harmful effects, due to their continuous introduction into it. In this study, an on-line solid phase extraction (on-line SPE) coupled with ultra-high performance liquid chromatography following by mass spectrometry detection (UHPLC-MS/MS)has been optimized to determine a group of natural and synthetic hormones (estrogens, androgens, progestogens and corticosteroids). The parameters that affect the extraction procedure (pH of the loading solvent, the sample volume and the composition of the wash-step) and the separation and detection process have been optimized. This method has been used for the determination of targethormones in samples of wastewater treatment plants from Gran Canaria (Spain) which use different water treatment methods. The results shown that several hormonal compounds were detected in the samples of wastewater treatment plants at concentrations of $ng \cdot L^{-1}$. **References:** [1] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli. The Challenge of Micropollutants in Aquatic Systems. Science. 313 (2006) 1072-1077 [2] P.B. Fayad, M. Prévost, S. Sauvé. On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry optimized for the analysis of steroid hormones in urban wastewaters. Talanda. 115 (2013) 349-360

WE146

NORMAN MassBank – status and new developments

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The web-based MassBank mass spectral database [1] (http://www.massbank.jp) allows the storage and searching (e.g. spectra, substructure and peak search) of any kind of high and low resoluton mass spectra including EI-MS, ESI-QToF-MSMS and ESI-FT-MS. MassBank was developed within the metabolimcs community of Japan and is now the official mass spectral database of Mass Spectrometry Society of Japan. Since 2010, the NORMAN association runs the NORMAN MassBank as the European mirror server of MassBank [2]. In terms of an easy generation of high-quality mass-spectra, the RMassBank package for R was developed to process spectra from Thermo LTQ-Orbitrap instruments [3]. In 2013, we elaborated on the integration of other instruments such time-of-flight analysers and Thermo QExactive. Furthermore, a validation tool for existing MassBank records was written and integrated in RMassBank to make quality control easier. References: [1] H. Horai, M. Arita, S. Kanaya, Y. Nihei, T. Ikeda, K. Suwa, Y. Ojima, K. Tanaka, S. Tanaka, K. Aoshima, Y. Oda, Y. Kakazu, M. Kusano, T. Tohge, F. Matsuda, Y. Sawada, M.Y. Hirai, H. Nakanishi, K. Ikeda, N. Akimoto, T. Maoka, H. Takahashi, T. Ara, N. Sakurai, H. Suzuki, D. Shibata, S. Neumann, T. Iida, K. Funatsu, F. Matsuura, T. Soga, R. Taguchi, K. Saito, T. Nishioka, Journal of Mass Spectrometry 45 (2010) 703. [2] T. Schulze, E. Schymanski, M. Stravs, S. Neumann, M. Krauss, H. Singer, C. Hug, C.M.J. Gallampois, J. Hollender, J. Slobodnik, W. Brack, NORMAN Bulletin 3 (2012) 9. [3] M.A. Stravs, E.L. Schymanski, H.P. Singer, J. Hollender, Journal of Mass Spectrometry 48 (2013) 89.

WE147

Source discrimination of priority and emerging pollutants in the framework of an immission cadastre for Luxembourgish surface waters

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CRP Henri Tudor has been commissioned with a study to establish an immission cadastre for priority and emerging substances in Luxembourgish surface waters. The approach consisted in an in-depth analysis of the appropriateness of the monitoring schemes in terms of hydrological representativeness (seasonality, discharge relation), speciation phase (liquid-solid) and limit of detections in order to be able to confirm non-detects and to evaluate sporadic detections of WFD priority pollutants. The remaining list of frequently detected substances has then been analysed for probable sources with the help of statistical data on economic activities and their waste profiles. In a second step an exploratory study has been launched to evaluate the presence of emerging pollutants in 14 river basins of different economic structure. The target compound groups were pharmaceuticals, biocides, flame retardants and surface treatment products. The collection of the pollutants has been performed by a POCIS passive sampler campaign of 2 weeks and a sediment net campaign of 3 days. The target compounds were analysed in the phase that was most appropriate. For compounds that had to be analysed by external labs no sampling rates were available for the POCIS calculations. Their concentrations are semi-quantitative. The evaluation of the data was based on the hypothesis that emissions related to economic activities could be distinguished from general consumption (pharmaceuticals, biocides) by generating correlations with the conservative drug Carbamazepine and observing outliers in the regression graphs. This procedure yields on one hand estimations of background pollution generated by general urban activities (living, housing transport) as related to population equivalents and on the other hand outliers pin-point specific emissions by economic actors.

WE148

Evaluation of alternative modeling approaches to estimate material-air partition ratios for semi-volatile organic chemicals.

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Semi-volatile organic chemicals (SVOCs) are used in a wide variety of consumer goods and throughout the industrial sector. Models of fate and transport of SVOCs in the indoor environment have been developed to describe exposure pathways to humans and explain variability of indoor concentrations. These models depend on partition ratios of SVOCs between various materials present in the indoor environment and air, which have only been measured for a few selected substances. Recently, models that employ Abraham solvation parameters to estimate material-air partition ratios ($K_{M/A}$) were proposed [1]. The models are empirical correlation between measured $K_{M/A}$ values collected from the literature and Abraham solvation parameters for the chemicals. We have developed alternative models by correlating measured *K*M/A values against pure liquid-air partition ratios ($K_{L/A}$). Our modeling approach would lead to a 1:1 correlation between $K_{M/A}$ and $K_{L/A}$ in the case that molecular interactions and energies of cavity formation in the pure liquid phase of the chemical are identical to those in the material. Deviations between our model and measurements are expected if there are intermolecular interactions (especially hydrogen bonds) between the SVOC and the material that do not occur in the pure liquid phase of the chemical, or where the enthalpy of cavity formation in the material differs from that of the pure liquid. To model $K_{L/A}$ values we employed experimental values for vapor pressure and liquid molar volume when available, but we also developed models based on estimated vapor pressure and molar volume to evaluate the robustness of our approach in cases where measurements are not available. We also employed an empirical correlation based on Trouton's rule to correct the measured $K_{M/A}$ values to a standard reference temperature of 298 K [2]. Our results demonstrate that correlations between $K_{L/A}$ and $K_{M/A}$ of SVOCs are as strong as those derived using the Abraham parameter approach. By adjusting $K_{M/A}$ values to a standard temperature we achieved much stronger correlations in both approaches than those previously reported [1]. Given the variability of these properties among the SVOCs examined, we view our model as promising enough to support future work in modeling organic chemical emissions from materials in consumer goods in the indoors based on partition ratios derived from vapor pressure. Holmgren et al, *Sci. Tot. Environ.* **2012** MacLeod et al, *Environ. Sci. Technol.* **2007**

WE149

Method optimization and analyses of organic environmental pollutants from meat processing industry wastewater

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Discharging of untreated wastewater has many negative effects on the environmental media (surface and underground water, sediment, air and soil) and human health, and is one of the main environmental issues in developing countries. The meat industry sector emerges as one of the most significant contributors to the serious environmental problems in Vojvodina region, Serbia. Wastewater generated during the meat processing is a complex mixture of compounds with a high load of organic matter as well as inorganic cations. Wastewater may contain pesticide residues, nitrogen, aerobic mesophile bacteria, total coliforms, faecal coliforms, E. coli and faecal streptococci, as well as presence of Salmonella sp. Very usefull and important parameter for observation is the phoshatase activity index which is used as a biochemical indicator of heterotrophic activity of aquatic microorganisms. Microbiological determination and differentiation as a part of holistic study approach for monitoring of ecosystem status are in progress. The development and optimization of sample preparation method (liquid-liquid extraction and evaporation) and analysis of meat processing industry wastewater will be presented. Optimization of the procedure was carried out by utilisation of different polarity solvents, in order to optimize the qualitative characteristics of extracts. During three sampling campaigns, eight samples of meat processing wastewater were collected from six companies. GC-MS instrument, Agilent 7890N GC, was selected and used in SCAN mode. The second goal of the research was identification of organic compounds with emphasis on hazardous and priority pollutants according to the EU Water Frame Directive 2000/60/EC (Annex V and VIII) and compounds from NORMAN list of emerging substances. The most frequently identified compounds were fatty acids, alcohols, detergents, antifoaming agents and branched and higher alkanes. Some compounds of special interest belonging to the groups of phthalates, benzenes, phenols and terpenes were also identified. According to the results of organic content residues the meat processing companies included in sampling campaigns are in different stages of in-situ industrial wastewater treatment implementation, which is defined by national legislation – from no treatment, to full operating plants.

WE150

Present and future emerging pollutant mixtures in European rivers predicted by use pattern emission modeling

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Predictive environmental risk assessment of chemical substances is seriously hampered by the lack of adequate release data as a source for exposure studies. On a European scale, the EU project SOLUTIONS develops a modeling procedure for aquatic exposure of the mixture of chemicals that are used and partly released into the environment. This methodology aims to cover the majority of chemicals used today as well as in the future, including industrial chemicals, pesticides,

pharmaceuticals and nano materials. The new emission modeling will be built as an extension to the SPIN tool providing data on substances in preparations used in the Nordic countries. As the most comprehensibly evaluated data set, the Nordic product registers contain valuable information on the actual use of chemical preparations and substances on the market both in terms of volumes, number of products, composition of products and the function and industrial categories where the substance can be found. The SPIN registry also contains estimates of use-associated emissions to all environmental compartments. As a first approximation, the Nordic use classification scheme and the resulting emission predictions are extrapolated to the European scale. After careful evaluation, the emissions serve as input to the SimpleBox model predictions of aquatic exposure concentrations and mixture impact (platform presentation “SimpleBox Solution”).

WE151

Using monitoring data to assess persistence, bioaccumulation and long-range transport

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Prospective environmental risk assessment is mainly based on data obtained in laboratory tests and on modelled exposure data. If chemical substances are already on the market and emitted into the environment, monitoring data become an additional source of information to assess their environmental fate and evaluate properties of high concern such as persistence, bioaccumulation and long-range transport potential. Closer coordination between the regulatory management of substances and environmental monitoring may allow validating or even revising regulatory decisions. Within the present project, existing chemical environmental monitoring programmes were evaluated with regard to the suitability of their results as additional information for environmental risk assessment. A number of German monitoring programmes as well as several international programmes providing information on long-range transport of chemicals were included in this evaluation. Monitoring data on concentrations of pollutants in sediments or biota over at least five years can be used to evaluate persistence. For human pharmaceuticals, i.e. polar substances that are mainly emitted into surface waters through wastewater treatment plants an approach was developed that allows a first rough classification of persistence. This concept can be applied to prioritise pharmaceuticals for further investigations (e.g. additional degradation tests). Data indicating long-range transport potential of substances can be attained from monitoring in the arctic environment and, if local pollution sources can be excluded, from monitoring of other remote areas like Alpine regions. Besides monitoring data, knowledge of the emission quantities and entry routes into the environment is necessary to draw conclusions on the persistence and long-range transport potential of a substance. If data on concentrations in organisms and their surrounding medium are available, bioaccumulation factors (BAF) can be calculated. Investigations on organisms belonging to different trophic levels of a food web allow the derivation of biomagnification factors (BMF) or trophic magnification factors (TMF). For PFOS, BMF were estimated based on biota concentration data of marine species samples retrieved from the German Environmental Specimen Bank. Since most of the evaluated programmes have been developed for other purposes, in many cases specific monitoring approaches will be necessary to provide data on substance properties of high concern.

Recent advances and critical future research directions for poly- and perfuorinated alkyl substances (PFASs) (P)

WE152

Screening and ranking of PFASs by MultiCriteria Decision Making approach in QSARINS for highlighting the priority compounds

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Poly- and Perfluorinated alkyl substances (PFASs) have been one of the classes of environmental pollutants studied in the recent FP7-EU Project CADASTER (Case studies on the Development and Application of in silico Techniques for Environmental hazard and Risk assessment). The interest on this class of chemicals was mainly due to the enormous lack of information for the majority of these compounds. Various physico-chemical properties and toxicity data were modeled by theoretical molecular descriptors to enlarge the data availability. The QSA(P)R models for PBT-Index, inhalation and oral toxicity in rat and mouse and for the prediction of vapor pressure and solubility in water, implemented in the new version of the software QSARINS, were applied to hundreds of PFASs included in the pre-registration list of REACH, verifying the belonging of each chemical to the Applicability Domain of the models. These new data availability for physico-chemical properties, useful for environmental distribution, PBT behavior and various toxicities can be used in screening and ranking approaches (such as the Multicriteria Decision Making approach (MCDM), implemented in the QSARINS-Chem module), to prioritize the most hazardous compounds, focusing the necessary experimental tests only on the highlighted PFASs.

WE153

The Environmental Profiles of 8:2 and 6:2 Fluorotelomer Alcohols

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The 8:2 and 6:2 fluorotelomer alcohols (1H, 1H, 2H, 2H-perfluorodecanol and 1H, 1H, 2H, 2H-perfluorooctanol, respectively) are important specialty chemical intermediates in the manufacture of various products. The perfluoroalkyl chain in these substances and their detection in environmental compartments (primarily air) have led to broad interest in their environmental fate as well as their mammalian and environmental toxicology. The ecotoxicity profile of the 8:2 fluorotelomer alcohol was previously discussed by Koch et al. (SETAC 2004) but little aquatic toxicity data have previously been presented on the 6:2 fluorotelomer alcohol. The acute and chronic aquatic data for the 6:2 fluorotelomer alcohol will be presented along with the presently known environmental fate data for the substance. Finally, a comparative evaluation of the environmental profiles of the two fluorotelomer alcohols will be discussed including their physical-chemical properties, environmental fate and effects, and the potential for bioconcentration/bioaccumulation in aquatic and terrestrial species with relevant implications for aquatic/terrestrial risk assessments.

WE154

Per- and Polyfluoroalkyl Substances (PFASs) in Swedish Rivers and the Baltic Sea

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Per- and polyfluoroalkyl substances (PFASs) are ubiquitous distributed in the environment. They are of concern due to their persistence and bioaccumulative and toxic properties. The aim of this study was to investigate the occurrence of PFASs in Swedish rivers and the Baltic Sea. Water samples were collected at 40 river sites along the Swedish coast (primarily at river mouths) and at 18 sampling sites in the Baltic Sea during September and October 2013. The samples were filtered through glass fiber filters (GFFs) and extracted using solid phase extraction (SPE). The analyses of PFASs were performed using high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) and HPLC coupled to quadrupole time of flight MS (QTOF-MS). Preliminary results show that the levels of PFASs vary depending on the water flow and anthropogenic impact on the rivers and the Baltic Sea.

WE155

Spatial distribution and partitioning behaviour of selected PFASs in surface water and sediment: a French nationwide survey

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The spatial distribution and partitioning of 22 selected poly- and perfluoroalkyl substances (PFASs) in 133 selected rivers and lakes was investigated at nationwide scale in mainland France. In view of the high number of data that fell below detection limits, statistical treatments taking into account left-censored data were implemented to compute descriptive statistics as well as correlations. On average, ΣPFASs amounted to 28 ng L^{-1} in the dissolved phase, and to 1.8 ng g^{-1} dw in the sediment. Although perfluorooctane sulfonate (PFOS) was found to be the prevalent compound on average in both compartments, considerable variations were observed between individual sampling stations, the most polluted stations exhibiting generally higher carboxylates (PFHxA, PFNA, PFTrDA) and 6:2 FTS levels. A Kohonen mapping was used as a preliminary step to identify similarities between stations as well as key correlations between PFASs compounds. Log K_d and log K_{oc} partition coefficients were also determined, and the correlation with the perfluoroalkyl chain length was modeled. Organic carbon fraction or grain size appeared to be significant controlling factors of PFAS accumulation in the sediment.

WE156

Characterization of the Perfluorinated Compounds Pattern in Mediterranean Aquatic Ecosystems: the Xúquer River as study case

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The composition and spatial distribution of various perfluorinated compounds (PFCs), comprising perfluorinated carboxylic acids (PFCAs), perfluorinated sulfonates (PFSs) and perfluorinated sulfonamides (PFSAs) were measured in, water, sediments and biota and coastal sediments from the Xúquer River Basin. This basin was designated as a European Pilot River Basin for the implementation of the Water Framework Directive and is located in the east of Spain. It has an area of 21,632km^2, a main stream length of approximately 500 km. This basin is affected by extreme flow events as part of the normal hydrologic behaviour, showing a consistent trend towards decreased discharge. Fifteen water and sediment samples and 25 fish samples were taken at different points of the river in 2010. From the 21 analytes included in this study, 12 were detected in water, 11 in sediment. Considering the three matrices perfluoroctane sulfonate (PFOS) and perfloropentanoic acid (PFPeA) were the most frequent PFCs. It is also remarkable the high frequency of perfluorobutanoic acid (PFBA) and perfluorobutane sulfonate (PFBS) in sediments, 100 and 98 % of the samples, respectively. Perfluoropentanoic acid was the PFCs found at highest concentration in biota (946.44 ng/g ww), perfluorobutane sulfonate in sediment (29.18 ng/g d.w.) and perfluorobutanoic acid in water (644.19 ng/L) showing the prevalence of short chain PFCs in all the samples. The log of the distribution constants (K_d or K_{oc}) in sediment and bioaccumulation factors (BAF) in biota ranged from 1.68 to 3.8 and from 4.45 to 6.28, respectively. Theses constants confirm only in part other reported results, probably because there are too many uncontrolled variables in a field study that can affect in an unexpected way. This finding needs further clarification in order to assess whether contamination is linked to “dot-like” pollutant release, which could explain the pattern.

WE157

Perfluorinated Organic Compounds in the Housatonic River Estuary and Long Island Sound

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Perfluorinated compounds (PFCs) are a class of emerging contaminants that are input into coastal waters by municipal and industrial effluents, non-point sources, and atmospheric deposition. This study focuses on the fate and transport of PFCs input by municipal wastewater treatment plant effluents (WTEs) to the Housatonic River Estuary (a major freshwater source to the Long Island Sound). New observations of PFCs in effluents as point source inputs to upstream river waters are coupled to a numerical model that predicts PFC concentrations within the Housatonic River Estuary and adjacent Long Island Sound. Model results are compared to new observations of PFCs in these coastal waters. Partition constants of PFCs to WTE particulate matter are also determined and used to predict sediment loadings. Results indicate most of the targeted PFCs behave as conservative tracers as they are mixed along the estuary. PFC concentrations are found in the Housatonic plume waters that sweep along the coast as they are transported and mixed by the Long Island Sound tidal flow.

WE158

Pharmacokinetics of Ten Perfluoroalkyl Acids (PFAAs) in Microminipigs: A New Experimental Animal for Chemical Studies

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Pigs have been used as animal models in numerous nonclinical trials because of their anatomical and physiological similarities to humans. However, the use of pigs can be attended by extra costs for large amounts of test materials and handling difficulties. In this oweverHstudy, we used a new minipig strain, the Microminipig (MMpig, Fuji Micra Inc., Shizuoka, Japan) to evaluate the pharmacokinetics of a mixture of 10 perfluoroalkyl acids (PFAAs): PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFDoDA, PFUnDA and PFOS. PFAAs are a group of emerging pollutants, and PFOS was recently listed as a persistent organic pollutant under the Stockholm Convention. MMpigs are the world’s smallest minipig with an average body weight around 7 kg at maturity. Here we fed three female MMpigs (aged 6–8 mos, body weight [b.w.] 9–14 kg) a single capsule filled with a mixture of 3 mg/kg b.w. of each of the 10 testing substances, while two similar animals were given a capsule without PFAAs. The daily feeding rate for 21 days of the experiment was 3% b.w., adjusted throughout this period. Blood samples (approx. 1–2 mL) were collected from the cranial vena cava in a time-dependent manner. After 21 days, all five animals were euthanized under deep anesthesia and dissected, and blood and organ samples were collected. All samples were stored at −20ºC. We extracted the PFAAs from tissues by using acetonitrile and a clean-up with WAX-SPE cartridges. The compounds were analyzed by HPLC/MS/MS. We found that the blood depuration of PFAAs (blood $t_{1/2}$), which we calculated with first-order elimination curves, were in the descending order of PFOS ≥ PFOA > PFNA > PFUnDA > PFDA > PFHpA > PFDoDA > PFBA > PFHxA > PFPeA. Among the eight body compartments analyzed, the liver was the greatest site of accumulation for PFOS and longer-chain perfluorinated acids, i.e., PFNA to PFDoDA. The PFNA body burden was the highest among the treated compounds, as 37% of the given PFNA dose was accumulated in tissues. Interestingly, we observed a trend for an increasing accumulation of perfluorinated acids in the brain with the increase in the number of carbon atoms in the compound. This was the first study to use MMpigs to elucidate the pharmacokinetics of a group of environmental pollutants. We found that MMpigs can be excellent experimental animals for toxicological studies due to their easy handling, cost efficacy for standards, and waste treatment.

WE159

Bioaccumulation of perfluorinated alkyl acids in bivalves of the Po river delta (Adriatic Sea)

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The river Po (north of Italy) is the major Italian river and in its basin fluoropolymer manufacturing facilities and industrial activities, where perfluorochemicals are widely used, are present. In 2006 it was recognized as the dominant source of PFOA in Europe. In the lagoons of the Po River delta the farming of molluscs is extensively developed. Consequently, the accumulation of these compounds in the aquatic trophic chain poses concern about the risks for end consumers, including humans. In this study an assessment of the contamination by perfluoroalkyl acids (PFAAs) of water, sediment, mussels and clams of the Sacca di Goro Lagoon located in the southern part of the Po River Delta was performed. Analysis of perfluorinated carboxylates (from C5 to C10) and perfluorinated sulfonates (C4 and C8) were carried out by LC-MS/MS coupled with on-line SPE for analysis of water samples and on-line turbulent flow chromatography for purification of the extracts of sediment and biota samples. PFPeA, PFHxA, PFHpA, PFBS and PFHxS were never detected in the mollusc samples. In mussels, the detected PFAAs had similar concentrations ranging from 0.10 to 0.41 ng/g ww. On the other hand, PFOA and PFOS were the dominant homologues in clams with concentration from 2.2 to 4.5 ng/g ww and from 0.59 to 1.13 ng/g ww for PFOA and PFOS, respectively. The pattern of contamination in clams and mussels agrees with the literature data on PFAS contamination in bivalves. Considering the Italian average daily consumption of fresh and frozen shellfish (4.6 g/person) there is no health risk arising from dietary exposure to PFOS and PFOA at levels found in mollusk collected in the Sacca di Goro lagoon of Po River Delta.

WE160

PFASs exposure routes for midges (Chironomus riparius): a field experiment

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Long chain perfluoroalkyl acids or sulfonates and a few precursors have been found in different media of the Rhone river downstream of Lyon (France). First results with *Chironomus riparius* exposed in laboratory to natural sediment (Rhone river) have shown that the midge bioaccumulate C_{11} to C_{14} carboxylic acids, perfluorooctane sulfonate (PFOS) and two precursors. Nevertheless, native chironomids present higher level of PFASs than chironomids exposed on laboratory. We hypothesize that these differences can be explained by the food added in laboratory, the sedimentary matrix structuration or the contaminants flows (fresh suspended matter (SM), overlying water). The objectives of this study were (i) to assess the accumulation and elimination kinetics of PFASs by midge larvae exposed to sediment cores under controlled conditions, (ii) to determine whether freshly deposited sediments or SM influence accumulation. For the first objective, natural sediment cores from a deposition site along Rhone river (France) downstream of an industrial site releasing amounts of PFASs were collected using polyvinylchloride (PVC) core closed with a polyethylene cork (PE) and stored at 4°C.Chironomids were exposed at their optimum temperature (21°C). Kinetics of uptake and depuration were released. PFASs were analyzed in water, pore water, sediment and organisms by LC-MS/MS. For the second objective, cages containing fourth instar larvae were exposed on the study site during 4 days. PFASs were analyzed in organisms. Presumably, this field approach will enable us to be closer to environmental reality. Results of both experiments will be presented and discussed, in order to elucidate the role of sediment fractions on PFASs accumulation at this site. The effects of temperature variations on caged organisms growth will be accounted for [1]. 1. Péry, A.R.R. and J. Garric, *Modelling effects of temperature and feeding level on the life cycle of the midge Chironomus riparius:*

An energy-based modelling approach. Hydrobiologia, 2006. **553**(1): p. 59-66.

WE161
Distribution and elimination parameters of PFASs using a physiologically-based pharmacokinetic (PBPK) model
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Elimination half-lives of PFASs in the human body are higher than those reported in other animal species, being hypothesized that there exists a resorption mechanism between urine and plasma. In this framework, the application of physiologically-based pharmacokinetic (PBPK) models is essential to understand the distribution of PFASs in the human body. The objective of the present work was to estimate the value of key parameters for a PBPK model applied as well as to validate this model to several PFASs. The PBPK model structure was developed by using the main target tissues for the PFAS distribution. Simulation results were obtained for 4 perfluoroalkane sulfonic acids: (PFBS, PFHxS, PFOS, and PFDS) and 7 perfluoroalkyl carboxylic acids (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA and PFTeA). Partition coefficients (Pk) were assessed by dividing the concentration of PFASs found in several autopsy tissues (liver, bone marrow, brain, lung, and kidney) by that in plasma. Experimental values were obtained from individuals who had lived in Tarragona area (NE of Spain). Elimination variables (Tm and Kt), as well as the free fraction, were obtained by adjusting the concentration values in the tissues according to the PBPK model described above. However, any difference in the distribution pattern according to PFAS length chain has not been found. Key parameters of a PBPK model, previously validated for PFOS and PFOA, have been estimated here. These data are going to be of great utility for assessing the time course distribution in the human body of PFASs, other than PFOS and PFOA. In this study, the elimination of PFASs was assumed to take place in the kidney, where there is a resorption of PFASs from the urine, back to the plasma. This mechanism seems to correctly describe the elimination of PFASs. No differences were reported in the distribution and elimination of PFASs upon application of the PBPK model, according to the length chain. However, further studies should focus on the role of the model uncertainty and its implications in the PFAS pharmacokinetics.

WE162
Influence of perfluorooctane sulphonate (PFOS) on the toxicity of emerging contaminants
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The concern for possible adverse effects on the environment of emerging contaminants, some of them classified as persistent organic pollutants (POPs), has grown in recent years. Perfluorochemicals represent one such group of contaminants and recent toxicological studies have focused on interactive effects with other compounds to identify an area where knowledge should increase. In the present study, we evaluated the influence of PFOS on the toxicity of three emerging contaminants (fluoxetine, galaxolide and 2-Ethylhexyl-4-methoxycinnamate (EHMC)) on freshwater algae, cladocera and fish embryos. These three compounds were selected for the broad range of lipofilicity, frequently observed occurrence in the aquatic environment, and toxicity to aquatic organisms. The effect-response curves of the three selected compounds were studied in each organism, combined with three PFOS concentration levels. This study was funded by Spanish projects RTA2010-00004 and CTM2010-19779-C02-01/02.

WE163
Emerging contaminants: Environmental Risk Assessment for soil organisms after biosolids application in agricultural land
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Biosolids application is considered an important input source of emerging organic pollutants in soil. Consequently, they should be taken into account when used for agricultural purposes and a primary concern should be the environmental potential risk for soil organisms; so, risk characterisation should be required. This study focused on knowing the presence of some emerging organic pollutants (polybromodiphenyl ethers, perfluorinated alkyl substances and dechlorane-related compounds) in Spanish biosolids. Thus, samples (composted / thermally-dried sewage sludges and composted municipal solid wastes) from 12 Spanish wastewater treatment or composting plants were analysed. The measured value ranges were: 31.36-1414.09 ng.g-1(∑PBDEs); nd-168.10 ng.g-1 (∑PFCs), and 2.28-51.77 ng.g-1(∑DP). To meet the objective, four exposure-assessment case studies were conducted by simulating the application of four biosolids in soil to assess the environmental risk of penta-BDE, deca-BDE, DP, PFOA and PFOS for soil organisms. Risk characterisation ratios indicate that after the first biosolid application in soil, the potential risk for soil organisms is relatively low (1.25E-06-6.40E-04) under the assayed conditions. This work was made possible by Spanish Government Grants CTM2010 19779-C02- C01/C02 and RTA2010-00004-C02.

WE164
Sublethal effects of Perfluorooctane sulfonate (PFOS) on Rainbow trout (Oncorhynchus mykiss)
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Perfluorooctane sulfonate (PFOS) is the most commonly found representative of perfluorinated compounds in the aquatic environment. Due to its physical-chemical characteristics, such as chemical and thermal stability, lipophobicity as well as water repellent properties, PFOS was widely used as surfactant in a variety of products. PFOS reaches surface waters mainly via discharges from municipal or industrial sewage treatment plants. Also direct entry into surface and ground waters by the earlier use of PFOS-containing fire-fighting foams contributed to a contamination of the aquatic environment. Only recently, PFOS was identified as priority hazardous substance in the field of water policy (EU, 2013). According to literature data, PFOS might influence population relevant endpoints such as reproduction, and endocrine functions. The aim of the present study was to examine, whether prolonged exposure of the common fresh water fish species rainbow trout to environmentally relevant concentrations of PFOS could influence endocrinological parameters. In a 28-day experiment 1.5 years old rainbow trout were exposed under flow-through conditions to nominal concentrations of 0.5, 1.0, 5.0, 10.0, 25.0, and 50.0 μg PFOS/l (potassium salt; CAS 2795-39-3). Control fish were maintained without any treatment in natural well water which was regularly checked for chemical and physical parameters. Real PFOS concentrations in the test aquaria were analysed once a week by HPLC-MS. After the exposure period histopathological investigations were performed. In addition, plasma concentrations of 17β-estradiol, testosterone, 11-keto-testosterone, triiodothyronine, thyroxine, and thyreoidea stimulating hormone were analyzed. Furthermore, the plasma concentrations of the egg yolk precursor protein vitellogenin, which is commonly used as a biomarker for estrogenicity of chemical compounds, were analyzed and set in context with sex steroid concentrations and gonadal maturity. The present study indicates that even exposure to 0.5 μg/L PFOS influences sex steroid and thyroid hormone levels. Predominantly female fish were affected. Since alterations were still observed in the lowest concentration tested (0.5 μg/l), the no observed effect concentration (NOEC) of PFOS is likely to be < 0.5 μg/l. These new data again confirm the currently specified environmental quality standard for PFOS of 0.65 ng/l (EU, 2013) to be appropriate.

WE165
Radiolytic decomposition of PFOS and toxicity assessment in aqueous media
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Perfluorinated compounds (PFCs) are persistent organic pollutants that cause adverse effects on human and wildlife. They are widely detected in aquatic environment such as river, lake and groundwater due to the low degradation capacity of conventional wastewater treatment process. Multilateral efforts are conducted with diverse advanced technologies to control the influence of PFCs on aquatic environment. However, little studies have been done with electron beam to improve the removal efficiencies of PFOS. In the present study, we investigate PFOS decomposition using electron beam at the absorbed dose of 0, 100, 300, 600 and 900 kGy. Three oxidants (persulfate ($S_2O_8^{2-}$), peroxymonosulfate (HSO_5^-) and hydrogen peroxide (H_2O_2)) are coupled with electron beam irradiation to increase the decomposition efficiency of PFOS with the concentration of 0.5, 1.0, 10 mM. The patterns of PFOS decomposition are monitored with liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS-MS). Each treatment is finally investigated with bioassays to confirm the adverse effect of by-products. Results show that the decomposition efficiency of PFOS by electron beam increases with increasing the absorbed dose and is 69.5% at an absorbed dose of 900 kGy. The addition of $S_2O_8^{2-}$ and HSO_5^- do not show any significant effect on decomposition of PFOS at the whole concentration range rather show decreasing tendency. PFOS are reported to rarely decompose with most of advanced oxidation process, thus electron beam can be used as alternative technology considering the removal rate at the absorbed dose of 900 kGy.

WE166
Mammalian toxicology of short-chain 6:2 fluorotelomer-based raw materials and degradation products
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The global fluorochemical industry is moving to short-chain perfluoroalkyl based products which have a move favorable toxicological and environmental profile. A key attribute of the short-chain substances is their rapid elimination in living systems. The change is driven by an industry commitment to curtail manufacture of long-chain perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate, perfluorohexane sulfonate and perfluorooctanoic acid and precursor products which may degrade to form them. These and other long-chain substances have been shown to have long elimination half lives in living systems. A comprehensive, robust mammalian toxicology database for 6:2 fluorotelomer-based raw materials including 6:2 fluorotelomer alcohol (6:2 FTOH) and 6:2 fluorotelomer methacrylate as well as key biotransformation products perfluorohexanoic acid (PFHxA) and 5:3 acid ($C_5F_{11}CH_2CH_2COOH$) has been developed. First tier studies included acute oral and dermal toxicity, single-dose uptake and elimination, genotoxicity and sensitization studies. Higher tier in-vivo studies followed and included repeated-dose toxicity and toxicokinetic assessments. This paper will present the results of these studies.

WE167
Mammalian and aquatic toxicology of short-chain 6:2 fluorotelomer-based commercial products
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The global fluorochemical industry is undergoing a significant change moving to short-chain perfluoroalkyl based products. The change is driven by an industry commitment to curtail manufacture of long-chain perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate, perfluorohexane sulfonate and perfluorooctanoic acid and precursor products which may degrade to form them. A comprehensive approach was undertaken to develop robust mammalian and aquatic toxicology data for 6:2 fluorotelomer-based commercial products. These products include polymers (e.g., acrylates and urethanes) and surfactant (e.g., phosphates, ethoxylates, sulfonates and betaines) products. Initial studies included physical-chemical property determinations, acute oral and dermal toxicity, genotoxicity and sensitization studies. Higher tier studies on substance classes followed and included repeated-dose toxicity assessments. This paper will describe the substances evaluated and the study results.

WE168
Anaerobic transformation of 10:2 fluorotelomer alcohol (10:2 FTOH) and 6:2 fluorotelomer sulfonate (6:2 FTS)
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Fluorotelomer alcohols (FTOHs) and fluorotelomer sulfonates (FTSs) are additives in a wide range of consumers- and industrial products. After use, many of these products are disposed in municipal landfills or in sewage sludge and sediments. However, the transformation rates of FTOHs and FTSs and formation of toxic metabolites such as perfluorocarboxylates under changing redox conditions are not well understood, due to the availability of limited studies that have investigated transformation rates under anaerobic conditions. Herein, we have investigated the transformation of 10:2 FTOH and 6:2 FTS under anaerobic/anoxic conditions. Manure was used as test matrix, in order to avoid interference from existing background levels of perfluorinated substances in sewage sludge. The experiment was performed in closed Erlenmeyer flasks at 24°C. During the 42 weeks incubation, a concentration reduction from 1035 ± 118 ng/g to 6 ± 2.3 ng/g wet weight was observed, corresponding to estimated concentration from 11496 ng/g to 66 ng/g dry weight or from 20.4 μmol/kg to 0.12 μmol/kg dw. Perfluorodecanoic acid (PFDcA) was found at the range of 89 - 144 ng/g dry weight (0.17 - 0.28 μmol/kg dw), accounting for 1.37 mol % of initial 10:2 FTOH. Detection of PFDcA shows that 10:2 FTOH can form the corresponding acid with similar carbon chain length. Thus, FTOHs can be a source for perfluorocarboxylates (PFCAs) under anaerobic/anoxic conditions in landfills, sludge or sediments. No significant degradation of 6:2 FTS was detected during the 42 weeks anaerobic incubation period.

WE170
Is perfluorooctane sulfonate (PFOS) biomagnified in riverine food webs? A case study of two French rivers.
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Perfluorooctane sulfonate (PFOS) might be the most widespread among per- and poly-fluoroalkyl substances currently identified in the aquatic environment. As such, it has been recently added to Annex B of the Stockholm Convention on persistent organic pollutants, and on the priority substances list in the context of the European Water Framework Directive (WFD). As a consequence, European member states will have to monitor PFOS in biota, and check water bodies' compliance with the corresponding environmental quality standard (EQS) for biota, which refer to fish. While several studies successfully demonstrated PFOS biomagnification in lacustrine or marine food webs, it is less certain that this process also occurs in (shorter) riverine food webs. We studied the distribution of PFOS and measured $\delta^{13}C$ and $d^{15}N$ in benthic invertebrates (5 to 7 taxa depending of the river) and two fish species, namely the barbel (*Barbus barbus*) and the chub (*Squalius cephalus*), from two French rivers, in order to assess whether or not this compound is biomagnified in the respective food webs. These fish species have been identified in France as potential biomonitors for most priority substances concerned by compliance checking on the basis of EQS for biota. If PFOS was biomagnified in these riverine food webs, and TMFs or BMFs could be determined, then it would be possible to monitor this chemical in benthic organisms and extrapolate to fish tissues, and thus limit the use of fish in monitoring. The results will be presented and discussed.

WE171
Bioaccumulation of perfluorinated alkyl acids in two benthic invertebrates: Lumbriculus variegatus and Chironomus riparius
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The aim of this work was to study the bioaccumulation of 3 perfluorinated alkyl acids (PFBS, PFOS and PFOA) in two benthic invertebrates: *Lumbriculus variegatus* and *Chironomus riparius*. Specimens of *Lumbriculus variegatus* and larvae of *Chironomus riparius* were exposed to microcosms with formulated sediment and water at nominal concentration of 10 μg/L. The distribution of the perfluorinated acids in the different compartments (overlaying water, sediment and organisms) at the end of the tests have been measured. Most of the added PFOA and PFBS were measured in the overlaying water (65%) whereas PFOS was mainly detected in the sediment (36 % of the added amount). No loss of PFOA was detected for evaporation while PFBS and PFOS were partially air stripped (25% and 45 % respectively) because of the aeration of the microcosms. Accumulation was higher in *L. variegatus* than in *C. riparius* for all the 3 perfluorinated alkyl acids. Accumulation in *C. riparius* were mainly due to uptake by aqueous phase: the bioaccumulation factors (BAF) based on water concentrations were 0.2, 3 and 19 $\mu g\ kg^{-1}\ ww/\mu g\ L^{-1}$ for PFBS, PFOA and PFOS respectively. On the contrary sediment ingestion was the main uptake route for *L. variegatus*: bioaccumulation is also dependent on the organic carbon content of the sediment that affects feeding rate of *L. variegatus*. The BAF based on sediment concentrations were 32, 55 and 47 $\mu g\ kg^{-1}ww/\mu g\ kg^{-1}dw$ for PFBS, PFOA and PFOS respectively. PFBS is potentially less bioaccumulable than its homologues with longer carbon chain because of its shorter carbon chain and its higher solubility. Nevertheless our results showed that, in sediment-dwelling organisms, bioaccumulation of PFBS can be of the same order of magnitude of that measured for PFOS. This result raises new questions about the environmental risk of this compound.

Biophysical Interactions at the Bio-nano Interface: Relevance for Aquatic Nanotoxicology (P)

WE173
Effect of sulfidation and natural organic matters on toxicity of AgNPs of sediment dwelling organism, Chironomus riparius
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Silver nanoparticles (AgNPs) readily transform in the environment, which modifies their properties and alters their fate and toxicity. Therefore to predict the environmental impact of AgNPs, the toxicity test should be conducted taken into account of their interaction with environmental matrices. Humic acid is known to mitigate AgNPs toxicity in natural systems and it is also known that silver binds strongly to sulfer and sulfidation of AgNPs results in significant decrease in their toxicity due to the lower solubility of silver sulfide, potentially limiting their short term environmental impact. However, little is known about the effect of potential competing natural ligands, such as, natural organic matters (NOM), which can prevent or slow down the sulfidation process. Moreover, though sulfidation of Ag surface has been intensively studied, little is known on its fate in sediment

compartment of aquatic environment and much less on its toxicity to sediment dwelling organism. To answer those questions, in this study, we investigated effect of sulfer on sediment dwelling organism, *Chironomus riparius* using acute and chronic ecotoxicity endpoints on relatively low exposure condition. We then investigated how NOM affect the effect of sulfer in toxicity of AgNPs in C.riparius. We also monitored Ag concentration in water and sediment compartment, as well as in *Chironomus* tissue with and without treatment of sulfer and NOM. Finally, to investigate how sulfer and NOM affect ion release from AgNPs, which is a crucial factor for determining AgNPs toxicity, we also monitored released Ag ion in each treatment. H2S mitigated AgNPs toxicity in both acute and chronic tests, however NOM did not affect acute toxicity of AgNPs in C.riparius. Results from complex exposure of sulfer and NOM with AgNPs will be presented in the conference.

WE174

Effects of dimercaptosuccinate-functionalized magnetic iron oxide nanoparticles on aquatic organisms

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Magnetic iron oxide nanoparticles (IONPs) have gained growing attention in recent years for their promising applications in medical treatments and environmental abatement. Among the IONPs, dimercaptosuccinic acid coated IONPs (DMSA-IONPs) are of great potentials because of their high internalization in cells and effective adsorption to heavy metals. Nevertheless, little is known on the effects of such particles towards aquatic organisms. The aims of the study were to (I) measure the stability of DMSA-IONPs and compare it to uncoated-IONPs (fresh and aged) in different test media; (II) measure and compare their toxicity using the growth inhibition test with freshwater algae *Pseudokirchneriella subcapitata* and duckweed *Lemna minor*, and the immobilization of water flea *Daphnia magna*. I) The stability was investigated after dispersion of DMSA-IONPs (100 mg Fe/L) in the three media used for biological testing. No agglomeration was found in algal medium even after 7 days and the Dynamic Size Distribution (DSD) measured by Dynamic Light Scattering (DLS) was constant; whereas in the media of lemna and daphnids, precipitates appeared already within hours. Uncoated-IONPs in the three media precipitated even faster that agglomeration started within minutes; II) The different test organisms were exposed to IONPs in concentrations ranging from 0.01 to 100 mg Fe /L. Inhibition of algal growth was observed for the three types of IONPs when the concentration of IONPs was higher than 1 mg Fe/L. The EC_{50} for DMSA-IONPs towards algae was 0.087 ± 0.012 mg Fe/L with no significant difference from uncoated-IONPs. Immobilization of daphnids occurred only in uncoated-IONPs. Neither DMSA- nor uncoated-IONPs significantly inhibited the growth of lemna. Compared to uncoated-IONPs the DMSA-IONPs were generally more stable in the test media. It can be assumed that the ionized and cross-linked DMSA molecules on the surface provide IONPs with high negative charges thus to prevent aggregation of IONPs in solution. In media that comparatively higher concentrations of Ca^{2+} and Mg^{2+} occurred (daphnids and lemna media), the stability of DMSA-IONPs decreased probably due to lowered interactions of the DMSA with the particle. Therefore, toxicity of DMSA-IONPs here is not necessarily related to the degree of stability; the different responses of the test organisms to DMSA-IONPs suggest that systematically ecological risk assessment on DMSA-IONPs application is needed.

WE175

ANTIOXIDANT RESPONSES AND HISTOLOGICAL CHANGES OF THE GILLS OF FRESHWATER FISH PROCHILODUS LINEATUS AFTER ACUTE AND CHRONIC EXPOSURE TO TITANIUM DIOXIDE NANOPARTICLES

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The toxic effects of titanium dioxide nanoparticles (TiO_2 NPs) in fishes, as well as their environmental concentration and dispersion in water bodies, are not yet fully established. The aimed of this study was to evaluate the toxicological potential of TiO_2 NPs and the mechanism of action of these NPs in the gills of *P. lineatus*. Then, the amount of reactive oxygen species (ROS), the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), the levels of glutathione (GSH) and lipid peroxide production (LPO), as well as the histopathological damage after acute (48 h) and chronic exposure (14 days) using 0, 1, 5, 10 e 50 mg L^{-1} TiO_2 NPs were evaluated. The LC50 - 48 h was > 290 mg L^{-1} TiO_2 NPs. After acute exposure to TiO_2 NPs the ROS levels decreased (exposure to 50 mg L^{-1} TiO_2 NPs) and the GSH levels increased (exposure to 10 and 50 mg L^{-1} TiO_2 NPs). After chronic exposure, ROS increased and SOD activity showed 26.16% of inhibition (exposure to 50 mg L^{-1} TiO_2 NPs), the levels of GSH increased after exposure to 5, 10 and 50 mg L^{-1} TiO_2 NPs. These results suggest that GSH play an important role in preventing oxidative stress in *P. lineatus* gills, since there was no lipid peroxidation. Histopathological indexes indicated normal gill function after acute exposure; however, the damages were classified as slight to moderate in animals exposed to 5, 10 and 50 mg L^{-1} TiO_2 NPs after chronic exposure. These data suggested that the TiO_2 NPs does not cause unbalance between pro-oxidants and antioxidants after acute and chronic exposure, but the histopathological alterations after chronic exposure may influence the exchange of gases and the osmotic and ionic equilibrium. Although the histological damages are reversible after decontamination of the aquatic environment, they may imply high metabolic costs to restore the gill epithelium. Financial support: Grant 2011/10339-0, São Paulo Research Foundation (FAPESP); CNPq/INCT-TA Proc. 573949/2008-5.

WE176

Uptake of gold nanoparticles in Daphnia magna in the presence and absence of food using electron microscopy

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The rapid increase in the use of engineered nanoparticles during the past decade underlines the importance of evaluating not only the acute toxicity of nanoparticles, but also their uptake and translocation in organisms. Transmission Electron Microscopy (TEM) is a well-established technique for imaging of biological samples e.g. for identification of changes to cellular structures and TEM is also widely used as a tool for identifying and characterizing nanoparticles In the present study the freshwater crustacean *Daphnia magna* was exposed for 24h to 0.4 mg Au/L citrate coated gold nanoparticles (Au NPs, 10 nm) in either the presence or absence of food (green algae, *Pseudokirchneriella subcapitata*). The uptake into gut lumen and internalization into epithelial cells of exposed *D. magna* was examined by light microscopy, TEM and FIB-SEM (Focused Ion Beam Scanning Electron Microscopy). No apparent disruption of gut cells was observed. The majority of Au NPs observed in the *D. magna* gut was located in the gut lumen both as single nanoparticles similar to stock solution and agglomerates/aggregates for both exposure scenarios. However, in the presence of food the amount of Au NPs in the gut-lumen seemed to decrease compared to treatment with absence of food. Few AuNPs were observed across the peritrophic membrane compared to in the gut-lumen. This suggests that the peritrophic membrane of the gut form a barrier with low permeability of the Au NPs. Only a few AuNPs were observed to have crossed the peritrophic membrane compared to the amount recovered in the gut-lumen. Occasionally, Au NPs were found inside gut epithelial cells indicating internalization. These results suggest that Au NPs are available for uptake into the gut-lumen both in the presence and absence of food. Additionally, it was found that the Au NPs can pass the PTM and to limit extend enter gut epithelial cells.

WE177

Biodegradable Polymeric Nanoparticles and Fungi

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Nanocapsule or nanosphere could be obtained from organic materials, including biodegradables such as poly-ε-caprolactone (PCL), and they have vast applications, including paint, toothpaste, UV protection, photocatalysis, photovoltaics, sensing, electrochromics, as well as photochromics. Nano and microencapsulation of natural compounds can lead to expansion of their activity, since encapsulation may increase the stability of the compounds and release them in the active center, allowing determining the mechanism of action and of interaction between insects and fungus. The toxicity for the environment caused by the active compounds and the nanocapsule constituents should not exist or to be below the toxic concentration. Fishes and fungi can be used to determine these toxicities, as well as the place of toxic action. Chrysophanol is one anthraquinone which present toxic effect to *leaf-cutting ant* (*Atta sexdens rubropilosa*), one of the most important agricultural plague insects and it do not present toxic action to aquatic organism such *Brachidanio rerio*. Nanoencapsulated chrysophanol was more toxic to ants. The present study assayed chrysophanol, nanocapsule or nanosphere of PLC empty or containing chrysophanol in order to determine the toxicity to *Leucoagaricus gongylophorus*, *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton rubrum*. The results of these assays and the development and validation of a specific analytical method using HPLC for quantification of the chrysophanol in biodegradable polymeric nanoparticles are presented in this communication. Poly-ε-caprolactone (PCL) was the polymeric matrix applied. The validation was performed by using a reverse-phase Nucleosil C18 column, a mobile phase consisting of THF:MeOH 7:3 (v/v), flow rate of 1.0 mL min-1 and UV-vis detector at 257 nm. The method has been applied in quality control program to develop new selective nanoformulations loaded with chrysophanol, which should be used in environmentally friendly program to control leaf-cutting ants. The higher encapsulation efficiency found in nanocapsules and nanospheres were 98.5% and 92.3%, respectively. The absolute recovery of chrysophanol in colloidal suspensions was nearly 100%. PCL nanocapsule and nanosphere do not present toxic effect to the fugi tested up to 100 μg/ml. Chrysophanol inhibit growth the *L. gongylophorus* in 21%. FAPESP, CNPq, CAPES, INCT-CBIP

WE178

Flow Cytometry as a tool to investigate the interactions between nanoparticles and aquatic microorganisms and their toxic effects at the cellular and subcellular level

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The interactions between ENPs and aquatic microorganisms are key in understanding the potential environmental hazards of ENPs. The application of Flow Cytometry (FCM) in ecotoxicology is rather recent and even more so in nanoecotoxicology. FCM can be a powerful tool for the investigation of interactions between nanoparticles and living cells as well as their toxic effects in complex media, but the systematic methodological approach is still missing. The objective of the present study is to assess the capabilities of the FCM to assess the sub-toxic effects of TiO_2 and CuO-NPs to the green microalga *Chlamydomons reinhardtii*. To this end the algae were exposed to 50 mg L^{-1} TiO_2 and 10 mg L^{-1} CuO-NPs in different exposure media (pH 5 – 8, ionic strengths 0.5 – 6mM) for 24h. Fluorescent molecular probes for cellular biomarkers of stress, such as Propidium Iodide for cell integrity, CellRox Green for general oxidative stress and CM-H2DCFDA for intracellular esterase activity. For each dye the contact time and dye concentrations were optimized. Optimization work was also performed to distinguish algal cell population from the ENPs aggregates of similar size and avoid possible artefacts. It was found that the differentiation between algae and ENP aggregates can be achieved by a definition of the appropriate gates in side (size and complexity) versus forward scatters (size) defined by using only ENPs and only algae runs on one hand and the chlorophyll autofluorescence of the algal cells versus non fluorescent aggregates on the other. In both conditions an increase in oxidative stress and membrane damage was observed but responses were more pronounced in the CuO exposure, which was attributed to the different mechanisms of toxicity, i.e. direct, contact-mediated (TiO_2) vs indirect ion-mediated (CuO). We demonstrate how TiO_2 and CuO NPs aggregates can be discerned from *Chlamydomonas reinhardtii* cells and how fluorescent probes can provide insight into the mechanisms of NP toxicity as a function of exposure conditions and can provide valuable insight into the dynamics of biological responses over time.

WE179

Preparation of Higher Plant and Phytoplankton Cells for the Study of Nanoparticle Uptake and Transport Using Electron Microscopy

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Uptake and transport of nanoparticles in plant cells are fascinating processes that are currently still not well understood. Transmission electron microscopy (TEM) would allow, due to its sub-nm resolution, unique insights into nanoparticle uptake mechanisms in plants cells at the cellular and subcellular level. Yet, TEM is often avoided because of high costs, time-consuming sample preparation involving highly toxic chemicals [1,2], and the (legitimate) concern of misinterpreting electron-dense artefacts as the nanoparticles under investigation. Here, we would like to share our experiences on the way to develop an abbreviated 7-day TEM protocol involving a reduced number of hazardous chemicals. Modifications will be presented to prepare land plants, aquatic plants, and phytoplankton exposed to various metal (oxide) nanoparticles, including examples of exposure in an environmentally relevant mesocosm scenario. Selected area X-ray diffraction as a way to validate composition of potential nanoparticles, and pitfalls, such as over- or underuse of staining agents, will be discussed. The resulting images provide direct evidence that nanoparticles >14 nm may enter plant/phytoplankton cells in as little as 6 hrs. Nanoparticles were clearly able to penetrate cellulose cell walls, which are currently assumed to be only permeable for nanoparticles [1] Hülskamp, M., Schwab, B., Grini, P., Schwarz, H. 2010. Transmission Electron Microscopy (TEM) of Plant Tissues. Cold Spring Harb Protoc 7:4958. [2] Wilson, SM, Bacic, A. 2012. Preparation of plant cells for transmission electron microscopy to optimize immunogold labeling of carbohydrate and protein epitopes. Nature Protocols 9:1716-1727. *Acknowledgement* - Study supported by the Swiss National Science Foundation. This material is also based upon work supported by the National Science Foundation (NSF) and the Environmental Protection Agency (EPA) under NSF Cooperative Agreement EF-0830093, Center for the Environmental Implications of NanoTechnology (CEINT) and TINE. Any opinions, findings, conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred.

WE180

Cytotoxicity assessment of graphene oxide (GO) and carboxyl graphene (CXYG) nanoplatelets – alone and in combination with aromatic environmental pollutants

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Graphene and graphene derivatives are a emerging class of carbon nanomaterials. Their increasing production and use in technical and consumer applications may be related with release of nano-sized graphene platelets into the environment resulting in human and environmental exposure. The objective of this study was to assess the intrinsic potential of graphene oxide (GO) and carboxyl graphene (CXYG) nanoplatelets to exert toxicity at the cellular level using the piscine hepatoma cell line PLHC-1 as experimental *in vitro* model. Furthermore we aimed to assess whether graphene nanoplatelets may act as vector for aromatic environmental pollutants, increasing their effective concentration inside the cell and thus their corresponding adverse effect(s). The cytotoxicity of GO and CXYG was assessed by a battery of assays measuring alterations in plasma membrane integrity, metabolic activity, and lysosomal and mitochondrial function. Induction of oxidative stress was assessed by measuring intracellular reactive oxygen species (ROS) levels. Their interaction with the cell surface, internalization and intracellular fate were studied by scanning and transmission electron microscopy. To assess possible combination effects between graphene nanomaterials and aromatic environmental pollutants, PLHC-1 cells were co-exposed to graphene nanoplatelets and an AhR agonist (β-naphthflavone (β-NF), benzo(k)fluoranthene (BkF) or 3,3',4,4',5,5'-Hexachlorobiphenyl (PCB169)). Subsequently, *cyp1A* mRNA expression levels and Cyp1A-dependent ethoxyresorufin-*O*-deethylase (EROD) activity were measured. It was observed that GO and CXYG nanoplatelets pierced through the plasma membrane. GO and CXYG accumulated in the cytosol of PLHC-1 cells, where they physically interacted with and damaged mitochondria. Mitochondrial damage was also evidenced by reduction in the mitochondrial membrane potential, and was associated with increased ROS levels. In the co-exposure experiments it was observed that the simultaneous presence of GO and CXYG nanoplatelets had a potentiating effect on β-NF, BkF and PCB169-dependent Cyp1A induction. This suggests that graphene nanoplatelets may have facilitated their entry into the cells, either by facilitating their passive diffusion by destabilizing/damaging the cells' plasma membrane (as evidenced by the previous results) and/or by a Trojan horse-like mechanism, that is, by binding them extracellularly and penetrating with them into the cytosol.

Environmental risk assessment of nanomaterials: open issues, pitfalls and recommendations (P)

WE181

Precautions in the ecotoxicity testing of silver nanoparticles: loss of ionic silver by adsorption and photoreduction over the test period

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According to The Project on Emerging Nanotechnologies, silver compounds are the most commonly found nanomaterial in the consumer market. Given their widespread availability, it is inevitable that they are released to the environment. However, the quantitative assessment of the toxicity (e.g. EC50) of silver nanoparticles (AgNPs) is still in its early stages and inconsistent across numerous studies. In the majority of studies, ionic silver (Ag^+) is shown to be the major factor for their observed toxicity. Consequently, accurate assessment of Ag^+ toxicity is of paramount importance in understanding the toxicity of Ag nanomaterials. It has been known for some time that silver in solution can adsorb strongly to different solid surfaces. Despite this knowledge, many studies to date have evaluated Ag and AgNPs toxicities without consideration for this effect, possibly due to the analytical challenges related to the accurate determination of Ag at the very low concentrations used in ecotoxicity testing. Here, we show that sorption effects occur also in the range of interest for Ag toxicity when following standard testing procedures on the model algal species, *Pseudokirchneriella subcapitata*. This raises a precautionary note in testing Ag and AgNPs due to their loss from solution via photoreduction and/or adsorption to container walls. The OECD freshwater alga growth inhibition test was conducted to study the toxicity of Ag (as $AgNO_3$) according to the OECD Guideline 201 for Testing of Chemicals. A parallel isotopic tracing experiment was conducted to monitor the changes in Ag solution

concentrations with ^{110m}Ag (as [^{110m}Ag] $AgNO_3$), which is a gamma-emitting isotope. Each solution containing Ag was tested under light (L) and dark (D) conditions, and aliquots were taken at t = 0, 24, 48 and 72 h. The results clearly show a decrease in the solution concentration of Ag by as much as 47 % at the relevant concentration, leading to an underestimation of the EC50 by over 90%. Additionally, a test with AgNPs also suggests an adsorptive effect for AgNPs that was dependent on surface functionality. The results presented in this paper flag an important warning to the nanotoxicology community, particularly in the study of AgNPs, as our results clearly indicate that a detailed monitoring of the adsorptive effects of Ag in ionic an nanoparticulate forms is essential.

WE182

Environmental Implications of Nanosilver – Case study to quantify the potential environmental risk associated with the exposure from nanosilver from textiles

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The application of nanosilver materials is supposed to have increased remarkably in the last years. Amongst other applications nanosilver is also introduced to textiles as antimicrobial agent. Nanosilver is a biological active substance, therefore it is mandatory to elucidate if an increase of nanosilver in the environment results in new risks to environmental organisms. The assessment of safety of nanomaterials is still an ongoing subject accompanied by shortcomings regarding missing exposure information or adequate scientific data on ecotoxicology and environmental fate. The aim of this study was to perform an exemplary environmental risk assessment (ERA) of nanosilver applied in textiles. Environmental exposure scenarios for nanosilver consider the washing of textiles in domestic homes and the release of wastewater to WWTPs. Data on ecotoxicological effects and environmental fate of the nanosilver NM300-K (JRC Nanomaterials (NM) Repository) as well as data on emission data for nanosilver from texiles were derived from a German joint research project called UMSICHT. The performed ERA was based on the specifications defined in the Technical Guidance Document II (2003). Concentrations, PECs and PNECs of nanosilver were deduced for sewage sludge and for the environmental compartments surface water, sediment and soil (after sewage sludge application). For the derivation of PECs different exposure approaches have been used considering the emission based on technological process data as well as emission based on tonnage data and default emission factors. Based on current shortcomings regarding valid quantitative information on production volumes, content of nanosilver in textiles as well as consumption rates of textiles equipped with nanosilver, all scenarios were chosen to be quite conservative. PNECs were from the effect values of the most sensitive organism for each selected compartment together with an appropriate assessment factor. The assessment factor depends on the quantitative and qualitative complexity of data for the selected nanosilver. Settings for the derivation of PECs and PNECs are presented and the obtained risk quotients are critically discussed. Shortcomings of the current data situation as well as missing, but mandatory information for a robust assessment were identified and recommendations how to improve the ERA of nanomaterials based on improved data are given.

WE183

Possibilities and limitations of the N-transformation test (OECD 216)

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Several guidelines are currently used to investigate the effects of chemicals on selected microbial activities under the framework of the REACH Directive. The procedure described in OECD TG 216 is used to assess the effects of chemicals on soil nitrifiers.The procedure described in OECD TG 216 is used to assess the effects of chemicals on soil nitrifiers. We investigated whether the applied nitrogen sources modify the effect on nitrification caused by two silver nanomaterials (differing in size and shape) and a soluble silver salt. We used three different test procedures: (i) nitrogen transformation using the complex organic nitrogen source lucerne (OECD TG 216), (ii) nitrogen transformation using the inorganic nitrogen source $(NH_4)_2SO_4$ and (iii) ammonium oxidation (ISO 15685). The results were compared with carbon transformation (OECD 217). The standard nitrogen transformation test using lucerne suggested that the test materials had no effect on soil nitrifiers, whereas significant effects were identified with the other two test procedures. The absence of effects with lucerne probably reflected the sorption of Ag+ to the additional organic nitrogen source thus reducing its bioavailability. This common test is therefore less suitable for the detection of effects caused by silver nanoparticles and soluble silver salts and we instead recommend the use of an inorganic nitrogen source or a test for potential ammonium oxidation. The observed effects were not specific to nanoparticles. The time course of the effect in both the nitrogen transformation test based on $(NH_4)_2SO_4$ and the potential ammonium oxidation test varied according to the test substance. This may be useful to determine the stability of silver nanomaterials, although further experiments are necessary to verify this hypothesis. Acknowledgement - The study was funded by the EU MARINA Framework 7 project.

WE184

Towards the development of improved OECD guidelines for the testing of nanomaterials - The OECD Expert Meeting on Ecotoxicology and Environmental Fate

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OECD test guidelines for the testing of chemicals are an important basis to assess potential hazards of anthropogenic substances to human and environmental health and assess their risks. As the guidelines were initially developed for the testing of conventional chemicals, their applicability to nanomaterials (NMs) is an area of debate. Hence, on behalf of the OECD Working Party on Manufactured Nanomaterials (WPMN) an expert meeting on ecotoxicology and environmental fate of nanomaterials took place in January 2013 in Berlin. At this meeting experts from science, industry and regulatory bodies discussed the applicability of selected OECD test guidelines (TGs) for the investigation of environmental fate and ecotoxicology of NMs. The objective was to discuss the current state of the knowledge and provide recommendations to the OECD WPMN on the need for (1) amending selected OECD TGs and developing new ones specific to NMs; and (2) providing guidance for the appropriate and valid testing of environmental fate and ecotoxicity endpoints for NMs. Experts at the meeting agreed that the majority of the discussed OECD TG were generally applicable for the testing of NMs, with the exception of TG 105 (water solubility) and 106 (adsorption-desorption). Additionally, aspects which need special attention regarding the application of OECD TG for the testing of NMs were highlighted (e.g., sample preparation, dispersion, analysis, dosimetry and characterisation). The recommendations of the expert meeting have led to the future development of proposals for new TG and guidance documents (GDs) to ensure that OECD TG give meaningful, repeatable, and accurate results when used for NMs. The poster will provide an overview on the main topics discussed during the meeting and the main outcomes. A more detailed report of the workshop will become available through the OECD. References: "Report of the OECD Expert Meeting on Ecotoxicology and Environmental Fate" (full report), 2013, submitted to OECD.Kühnel D. & Nickel C. "The OECD Expert Meeting on Ecotoxicology and Environmental Fate – Towards the development of improved OECD guidelines for the testing of nanomaterials" Science of the Total Environment, short communication, *accepted*. Keywords: risk assessment, OECD Working Party on Manufactured Nanomaterials (WPMN), OECD test guideline, guidance document

WE185

The DaNa Knowledge Base Nanomaterials - Latest research results on the effects of nanomaterials on humans and the environment

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Nanotechnology is considered one of the key technologies of the 21st century. The success of this fascinating technology is particularly based on its versatility. It will bring about fundamental changes of basic research as well as many sectors of industry and of life from electronics to the health care system. However, many consumers miss reliable and understandable information on nanomaterials and nanotechnology, e.g. on the basic questions: *What exactly are nanoparticles? What is meant by "exposure"? When do toxicologists speak of a risk?* These and many more questions are answered by our web-based knowledge base: **www.nanoobjects.info**. In an interdisciplinary approach scientists of the DaNa expert team provide a knowledge base for more transparency wrapping up the results of current research on nanomaterials regarding their influence on humans and the environment in an understandable way. Our presentation of complex scientific data addresses not only the scientific community but is also intended for the broader public, e.g. consumers, journalists, students or scientists from other research areas. Information on existing applications, material properties, and exposure routes as well as human and environmental toxicology are also provided and can be accessed either via the integrated application-based database or via the menu toolbar organised according to the different types of nanomaterials. In order to facilitate the evaluation process of scientific publications a methodology was developed in accordance with quality criteria that have been acknowledged worldwide within the scientific community. Furthermore, the DaNa-project acts as an umbrella project for current projects funded by the BMBF (German Federal Ministry of Education and Research) with regards to nanotechnology and their ecological benefits. Thus the collected knowledge integrated into the DaNa database could also contribute to the prioritisation of further research needs. DaNa is also on Twitter: **@nano_info**. Reference: Marquardt, C. et al. (2013) *Latest research results on the effects of nanomaterials on humans and the environment: DaNa – Knowledge Base Nanomaterials*. Journal of Physics: Conference Series 429(1): 012060. DOI: 10.1088/1742-6596/429/1/012060. Keywords: knowledge base, engineered nanomaterials, toxicology, background

WE186

Using Species Sensitive Distribution to assess and compare the toxicity of Silver nanoparticles and its bulk material through an aquatic trophic chain

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In the 21st century, nanotechnology has been considered one of the most promising fields of science. Different nanoparticles have been incorporated in household products such as clothes, food, toys, and personal care products. Silver nanoparticles, for instance, are the most used nanoparticles worldwide due to its biocidal proprieties. In the present study, Sensitive Distributions Curves (SSDs) were used to estimate the hazardous concentrations of silver nanoparticles and silver ions to species belonging to different trophic levels in aquatic ecosystems. The SSDs curves were built for microbial decomposers, primary producers, primary consumers, and secondary consumers using effective concentrations, LC_{50} and EC_{50}, from short-term toxicity tests. The hazardous concentrations to 5 and 50% of the species are calculated. The results from the SDDs were compared and discussed. Briefly, a comparison between the data from nanoparticles and bulk materials showed a higher toxicity of silver ions. Moreover, both compounds were more toxic to the organisms from the low levels of the trophic chain indicating that once in the environment both silver ions and nanoparticles might provoke a bottom-up effect in aquatic ecosystems.

WE187

Biological Activity of Magnetite-Humics Nanocomposite in Biotest-Systems

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This paper summarizes a set of bioassays using an magnetite nanoparticle-humic acids composite (Fe_3O_4-HA) which has high sorption properties with respect to ions of Pb^{2+}, Cd^{2+} and UO_2^{2+} ions [1, 2]. The main toxicological characteristics of the Fe_3O_4-HA, as well its biological activity in terms of the concentration limits for the use of these materials were evaluated. The effect of the nanocomposite on cells of various test organisms varied markedly. Based on biological response to the same concentration of the nanocomposite, species may be arranged in decreasing sensitivity as follows: *Scenedesmus quadricauda* > *Sinapis alba* > *Bos taurustaurus*,and the infusorians *Paramecium caudatum* exhibited no toxicity at the two lowest assessed concentrations. Fe_3O_4-HA concentration in 0.001% was absolutely safe for all test organisms; the range from 0.001 to 0.01% was still safe for higher plants and bull spermatozoa but toxic for algal cells which appeared to be the most sensitive to Fe_3O_4-HA. Further concentration increases up to 0.1% and 1.0% was toxic for the whole battery of organisms. Experiments with this "battery" of four biotests showed that, in controlled chemical conditions, water suspensions of the preparation, can be used safely for biota only up to a certain concentration limit. Probably, in natural conditions the presence of organic material, especially of humic substances, may contribute to an increase in the permissible concentration limit and reduce the harmful effect of the nanocomposite sorbent. It is obvious that before applying such remediation under specific biotope conditions, the biosafety of the composite should be assessed with a similar biotesting battery. Acknowledgements. This work is supported by the ISTC grant (project KR-2092). Reference: Pomogailo AD et al. Magnetoactive humics-based nanocomposites. *Macromol. Symp.* ***304*** (2011) *18-23*. Yurishcheva AA et al. Sorption of Pb^{2+} by magnetite coated by humic acids. *J. Biol. Physics and Chemistry* **13** (2013) 61–68.

WE188

Improving Environmental Flow Modelling for Exposure Assessment of MNM – Implications for a Time Dynamic Modelling Approach

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In recent years manufactured nanomaterials (MNM) were applied in a growing number of products. Once released to the environment these materials pose a potential hazard to humans and ecosystems. To assess the risks arising from these materials, their exposure to humans and ecosystems has to be determined. For nanomaterials a direct measurement of environmental concentrations is not feasible yet. Instead, material flow modeling provides a methodology for an indirect assessment by investigating the environmental path of the material. Available information about these flows is usually incomplete. The use of Bayesian models and the representation of uncertainty about the true value of a system parameter as marginal distribution provide an explicit representation of the existing system knowledge and its limitations. With that the model outcome also comprises and displays the enclosed assumptions and thus leads to more credible and better assessable simulation results. The Probabilistic Material Flow Analysis (PFMA) methodology combines both approaches and enables an indirect exposure assessment that explicitly represents and processes uncertain knowledge. PMFA computes a steady state of a system of dependent flows and evaluates arising material accumulations. So far, it is the only modeling approach used for an exposure assessment of NMN. However, some processes that lead to a local accumulation of MNM and thus exposure are not immediate. They comprise intermediate storage in certain parts of the system and time delays between flows and before the release of material. Their representation as immediate flows in the model could be inadequate. A time dynamic modeling and simulation approach would lead to more appropriate results. Based on an example scenario of the life cycle of MNM applied in façade coatings requirements for a dynamic modeling approach are derived. These requirements are discussed in the light of the original PMFA and from that implications for a new dynamic probabilistic material flow model approach are made. Finally the new approach is outlined.

WE189

Comprehensive Probabilistic Modelling of Environmental Emissions of Engineered Nanomaterials

T. Sun,

Currently very little is known about engineered nanomaterial (ENM) concentrations in the environment. In 2009, we have reported the first environmental concentrations for different ENM in various environmental compartments[1] by applying probabilistic material flow modelling. The present study aims to present more comprehensive and up to date environmental concentrations of ENM. The analysis was conducted in two stages: (1) modelling updated material flows and environmental concentrations of five ENM (nano-TiO_2, nano-ZnO, nano-Ag, Carbon Nanotubes (CNT) and Fullerenes (C_{60})); (2) modelling the flow and environmental concentration of TiO_2 pigment and reviewing the literature on concentrations of measured TiO_2 and total zinc and silver metal. Current ENM production estimates are generally higher than a few years ago. This indicates a rapid development of the nanotechnology sector. An exception was for nano-Ag, its production in 2012 was modelled to be lower than the assumption from 2009, reflecting a better knowledge on production amounts of nano-Ag. The newly predicted concentrations of ENM were in general 1 to 70 times higher, mainly due to the larger production in our new evaluation. Comparison between concentrations of ENM and their total materials were made in four compartments: sewage effluent, surface water, sediments and soils. They were chosen because first they are very relevant compartments; secondly there is relatively much concentration information available about the studied materials. Overall, the concentrations of total materials are 1 to 7 orders of magnitudes higher than that of ENM, except the measured TiO_2 concentration in STP effluent and in surface water. The measured concentration of TiO_2 in surface water was around the same levels as those modelled for TiO_2 pigment, which validates our modelled results well. Concentrations of total Zinc are around two orders of magnitudes higher than that of modelled nano-ZnO. Exception is the concentration in soil, where the concentration discrepancy between nano and total varies about 7 orders of magnitudes. This can be explained by the high natural background of total Zinc and the instable environmental property of nano-ZnO. When it come to the case of the comparison between nano-Ag and total Ag, the results among different compartments look more homogenous, concentrations of nano-Ag are between 2 and 3 orders of magnitudes lower compared to that of total Ag.

WE190

Flows of engineered nanomaterials through the recycling process in Switzerland

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Nanotechnology is a field of increasing interest for industry, academy and society in general, because it potentially represents an outbreak in human development due to its expected contributions in diverse sectors. Each year increasing amounts of engineered nanomaterials (ENMs) are produced, and more and more applications are launched to the market. As ENMs increase their interaction with human-environment systems, there is an increasing concern on the negative effects that they could have, as long as ENM properties are still poorly understood. The goal of this work was to determine the fate of nano-TiO_2, nano-ZnO, nano-Ag and CNT in Switzerland after the products containing them enter into the recycling system. Using a Probabilistic Material Flow Analysis (PMFA) approach, the fate of ENMs in the recycling system was determined. This was achieved by evaluating those recycling processes that are relevant for today's nano-enabled products. The results show that the largest fraction of ENMs are transferred during the recycling processes to the waste incineration plant and landfills. Only very small amounts were identified to flow back to the productive process of the economy. Smaller amounts of ENMs are transferred to new products, and some fractions are exported. Overall the results of the modeling show that recycling processes do not result in significant further dissipation of ENM to other products but that they are most likely ending up in waste fractions that can be properly handled.

WE191

Adequacy of current regulatory assessment for nanopesticides
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Applications of nanoformulations within the agrochemical sector are only just emerging, and many predict a rapid growth in coming years. The anticipated new or enhanced activity of nanopesticides will inevitably result in both new risks and new benefits to human and environmental health. It is unclear whether the current regulatory framework is adequate for the evaluation of these new products. A literature review [1] was carried out with the objectives (i) to explore potential applications of nanotechnology within the pesticide formulation sector, (ii) to identify possible impacts on environmental fate, and (iii) to analyse the suitability of current exposure assessment procedures to account for their novel properties within the EU regulatory context. A variety of sources were extensively searched and relevant information was combined from published literature, company websites, patent databases, reports from governmental and non-governmental institutions. The latest trends in research [2] indicate that polymer-based nanoformulations seem to have the greatest potential for further development, followed by formulations containing inorganic nanoparticles (e.g., silica, titanium dioxide) and nanoemulsions. Investigations into the environmental fate of nanopesticides remain scarce, however, and the current state of knowledge does not appear to be sufficient for a reliable assessment to be made of the benefits and risks associated with nanopesticides. With the aim to address the knowledge gap, experiments were carried out on a series of polymer-based nanopesticides. The suitability of standard regulatory protocols to determine fate parameters in soils (OECD tests for sorption and degradation) was evaluated in the context of pesticide regulatory assessment in the EU. Discrepancies between free and nanoformulated active ingredient were also analysed based on the results obtained by more realistic experimental set up and from the characterization of the nanocarriers. Results serve as a useful basis to discuss the (in)adequacy of current protocols, and identify priorities for research. [1] Kah M et al., 2013. Nano-pesticides: state of knowledge, environmental fate and exposure modeling. Critical Reviews of Environmental Science and Technology. 43, 1823-1867. DOI:10.1080/10643389.2012.671750 [2] Kah M and Hofmann T 2014. Nanopesticides research: current trends and future priorities. Environment International In press. DOI: 10.1016/j.envint.2013.11.015

Bioaccumulation processes and mechanisms: Implications for experimental assessments and modelling (P)

WE192
A weight-of-evidence (WoE) approach for evaluating trophic magnification in the environment.
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The potential of a chemical to accumulate in living organisms and increase in concentration with increasing trophic level within a food web are important considerations for assessing ecological risk. Trophic magnification factors (TMF), which describe the increase in concentration of chemicals in organisms that occupy successively higher trophic levels within a food web, are increasingly being used to assess bioaccumulation of chemicals in the environment. Because they are not conducted following an established guideline, there is a need to assess field-based TMF studies for their quality and comparability across diverse study areas and environments. Weight-of Evidence (WoE) offers a consistent, objective, and transparent appraoch for assessing TMF studies so that they may be appropriately included into a risk assessment. Quantitative WoE methods will be applied to available TMF studies on cyclic volatile methylsiloxanes (cVMS) in order to evaluate the impact of experimental design, environment, food web structure, species composition, and exposure on the relative utility of these studies for risk assessment. Probabilistic methods will also be used to determine how variations in these parameters influence the results and uncertainty associated with the studies.

WE193
Comparison of trophic magnification factors (TMFs) based on trophic level positions derived from stable isotope signatures to TMFs based on trophic level positions obtained from FishBase.
D.E. Powell, Dow Corning Corporation / Health Environmental Sciences; D.C. Muir, Environment Canada / Water Science and Technology Directorate; R.M. Seston, K.B. Woodburn, Dow Corning Corporation / Health Environmental Sciences
The potential of a chemical to accumulate in living organisms and increase in concentration with increasing trophic level within a food web are important considerations for assessing ecological risk. Trophic magnification factors (TMF), which describe the increase in concentration of chemicals in organisms that occupy successively higher trophic levels within a food web, are increasingly being used to assess bioaccumulation of chemicals in the environment. The field-based TMF is typically quantified using stable isotope ratios of nitrogen (N; δ^{15}N), which are used as a continuous variable for estimating the trophic level position occupied by each organism in the food web. Interpretation of trophic level position is based on the assumption that a constant enrichment of the heavier isotope occurs with each trophic level step. An expectation of this assumption is that all consumers in the food web have a constant δ^{15}N signature at the base of the food chain on which they are feeding. Omnivorous feeding across food chains and migration of consumers across large study areas having variable δ^{15}N signatures may skew interpretation of trophic level position. Similarly, δ^{15}N signatures of short lived or young low trophic forage level species are more susceptible to natural, catastrophic, or pollution induced fluctuations of δ^{15}N than the longer lived high trophic level predatory species. A possible method that may be used to correct for these biases and to validate results would be by direct comparison of trophic level positions derived from δ^{15}N signatures to trophic level positions obtained from FishBase, which was developed at the World Fish Center in collaboration with the Food and Agriculture Organization of the United Nations and support from the European Commission. This presentation will compare TMFs based on trophic level positions derived from δ^{15}N signatures to TMFs based on trophic level positions obtained from FishBase for select chemicals from across multiple food webs. Probabilistic methods will be used to evaluate the level of uncertainty associated with the comparisons.

WE194
Gastrointestinal absorption processes of substances: Do they have an impact on bioaccumulation?
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According to experiences in bioaccumulation studies in fish it is known that not for all classes of substances the bioaccumulation potential can be explained by their lipophilicity / hydrophobicity. The aim is to elaborate a strategy for identifying substances with increased bioaccumulation by absorption processes in the gastrointestinal tract (GIT). Therefore, accumulation processes in the GIT have been evaluated to get a first impression of the gastrointestinal absorption behavior of molecules. A compilation of different transport mechanisms, transporter types and the occurrences in different species will form a basis for discussion of the described effects. Especially for pharmaceuticals the caco-2 assay (human carcinogenic intestinal epithelium) is often used to investigate uptake processes in the GIT. Since this cell line represent both active and passive transport processes, the possibility to use the caco-2 cell line for prediction of increased bioaccumulation is examined. Therefore several measured and estimated caco-2 data-sets were plotted against logD (log Kow). In addition, the correlation to experimental bioaccumulation values was verified. In a first approach BCF values have been used as not enough BMF values are available. In the GIT different absorption mechanisms are relevant: passive permeability and active transport (primary and secondary). While passive uptake can often be predicted from molecule specific properties (log Kow, MW, charge) the secondary active transport enables also e.g. highly water-soluble substances to resolve membranes. Therefore, secondary active transport processes such as PEPT1, which is distributed throughout different animal species and humans, might lead to an underestimation of molecules ability to account for higher BCF values than predicted. First results indicate that data from caco-2 cell assays can indeed give some information for substances with probably higher bioaccumulation potential than predicted by the conventional approach based on lipophilicity. Thus, caco-2 data could form a basis to develop an estimation tool for identifying such substances. However, at first, the data base should be extended by experimental caco-2 values for selected industry chemicals, pesticides or biocides. As a perspective the estimation tool could be integrated in decision trees for bioaccumulation testing in a regulatory context (e.g. REACh).

WE195
Modelling the Passive Membrane Permeability for neutral molecules using experimental blood-brain barrier (BBB) data
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The BBB restricts the exchange of chemicals between blood and brain and is of central importance for pharmacological studies and environmental fate assessment in organisms alike. In this study we derived and tested a mechanistic permeability model for the BBB that is only based on molecular structure. Experimental permeation data from a saline buffer at pH 7.4 in rats and mice from literature have been used for validation, because these data are based on a highly standardized in-situ brain perfusion method where side aspects like metabolism can be ruled out. Great care has been taken, that only passive permeation data without active transport were considered. The permeation depends on diffusion coefficients through the membrane, equilibrium partition coefficients to the membrane and the

diffusion path length - in recent publications this has been modelled with a ppLFER (poly parameter linear free energy relationship) approach, although this should intrinsically not be applicable to a combination of diffusion and partitioning. Here the aim goes towards a mechanistic understanding of permeability aiming at a priori predictions which might later be used for bigger picture models like PBPK (physiologically based pharmacokinetic) modelling of whole organisms. Therefore the BBB is described as consecutive layers of resistances that are made up of the aqueous boundary layer (ABL) followed by the membrane. Since the membrane consists of a hydrophilic headgroup and an interior of hydrocarbon chains the total system can be simplified to the most extreme heterogeneity one can think of here: an aqueous layer followed by a hexadecane layer. This should not shrink the complexity of the system in regard to the initial question, but facilitate efficient modelling. It is justified since the membrane headgroup cannot be as extremely hydrophilic as water itself – thus the polar part of the membrane cannot play a role in the permeation process. Thus for the total permeability it can be concluded that the hydrophobic compounds whose permeability is dominated by the water layer should have quite similar permeabilities and that their remaining variance should depend on their aqueous diffusivity. For hydrophilic molecules on the other hand the permeability should scale with their hexadecane/water partitioning - while the borderline depends on the thickness of the ABL.

WE196
Determination of partition coefficients of ionogenic organic chemicals to phospholipid membranes in TRANSIL system
Y. Chen, Institute for Risk Assessment Sciences / Institute for Risk Assessment Sciences; J.L. Hermens, Utrecht University / Environmental Toxicology; s.T. droge, Utrecht University / IRAS
It is estimated that nearly 50% of the chemicals registered under REACH are ionogenic or permanently charged in the environment. The chemicals represent pharmaceuticals, surfactants, personal care products and biocides etc. The understanding and the development of predictive models of bioaccumulation for ionogenic organic chemicals (IOCs) is not as well established as that for neutral organic chemicals. Recently a bioconcentration model for IOCs in fish has been developed by Amitage et al. (Environ. Toxicol. Chem. 2013, 32, 115-128) and evaluated against empirical bioconcentration factors (BCFs). Modeled BCFs are well correlated with most observed BCFs. One of the key reasons for outliers in the BCF-IOC model is related to the uncertainties in estimating membrane affinity of IOCs. Charged IOCs tend to interact with polar groups and less with non-polar tail on phospholipid membrane. In this study, we have selected a series of acids and bases from a BCF database. These IOCs show considerable differences in structure (e.g. acidic functional group or amine type), empirical BCF values and estimated biotransformation rate constant. Solid-supported lipid membranes (TRANSIL) has been applied to determine partition coefficientsof these selected IOCs at a fixed of pH 7.4, where more than 99% of IOCs are present in charged form. The effect of difference in molecular structure on membrane affinity was also investigated.

WE197
The fish eyes as new target organ for trace elements accumulation - a new insight on environmental risk assessment
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Numerous works quantified contaminants in fish organs as a mean to evaluate environmental quality, seeking for causal relationships with animals' health. Several works pointed that liver and kidney are the best choices due to their roles in fish metabolism, while gills demonstrated their relevance associated with their direct contact with the surrounding environment. Fish eye is also in contact with water and thus could be able to uptake trace elements. Additionally, it has a key role as a neurosensory structure, collecting and focusing images and transforming them into neural signals. Nevertheless, there are few studies associating environmental data with accumulation levels in fish eyes. To fill this knowledge gap, an investigative biomonitoring study was carried out in two Portuguese estuaries with different contamination profiles. Tagus estuary has a confined area (Barreiro) that is severely contaminated by several trace elements, particularly Hg, Pb and As, while Aveiro lagoon is mainly contaminated by Hg in its upstream area (Laranjo). Juveniles of the golden grey mullet (*L. aurata*) were collected in the Tagus estuary and Aveiro lagoon in winter 2011 and winter 2013, respectively. Fish were captured in the most contaminated area of each estuary, as well as in a respective reference site. Water and sediment characterization confirmed that Barreiro and Laranjo are highly contaminated areas. Accordingly, fish eyes accumulated higher levels of trace elements at those contaminated areas. These findings support the use of fish eyes as a target organ in environmental health assessment since they faithfully reflect sediment and water contamination.

WE198
The Relevance of Soil for Human Exposure to PCDD/F
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Polychlorinated Dibenzo-p-dioxins and -furans (PCDD/F) are ubiquitously distributed due to their formation as unwanted by-products in a number of processes. They show a high toxicological potency in animals and humans and the most toxic PCDD/F congener has meanwhile been classified to be carcinogenic to humans by the IARC. Human exposure to dioxins is possible via several routes, where the exposure via dietary intake of food is known to be the most important pathway resulting in more than 90% intake. Nevertheless, soil can play an important role for the exposure of humans to dioxins. This is true for *direct* exposure by soil ingestion which can be expected to be up to a few hundred milligrams per day. In case of highly contaminated dust (like in contaminated living areas) or soil in contaminated areas this pathway can be highly relevant. The influence of contaminated soil can be even more pronounced by *indirect* exposure via the consumption of contaminated food. Animals like cows, buffalos, sheep and poultry can have a high uptake of soil during feed intake. The uptake of soil by grazing animals can be up to 20 % in weight of the daily intake. This intake of soil can be even higher when looking at chicken. For free ranging chicken an uptake of soil has been observed to be at more than 40 %. Some years ago, an incident of illegal open waste burning in the Region of Campania (Naples, Italy) caused a large-scale contamination of buffalo milk and milk products used for the production of Mozzarella cheese. During the investigation it could be demonstrated that the feed and soil concentrations are responsible for the high levels of dioxins found in the milk with 26.6 % of 732 buffalo milk samples analysed in our laboratory being above the European legislative maximum limit for PCDD/F-TEQ. A calculation concerning the effects of soil uptake by buffalos, assuming daily soil intake, milk production and taking into account the carry-over rates for cows is shown. It can be demonstrated that even inconspicuous soil concentrations of PCDD/F, not surpassing environmental control levels, can contribute considerably to the contamination of buffalo milk in terms of pushing levels towards the EU limit values. This might be a considerable factor contributing to the total human PCDD/F intake.

WE199
Predicting bioaccumulation of chemicals in worm – moving beyond chemical partitioning
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Chemical partitioning has been the starting point for estimating bioaccumulation potentials of contaminants in various biological species. For instance, bioconcentration of highly hydrophobic organic compounds in fish can be sufficiently modeled considering solely the partitioning between the target biota and the aqueous medium (i.e., the exposure phase). Similar approach has been applied to explain bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) of organic compounds in oligochaete (e.g., Connell & Markwell 1990 *Chemosphere* 20:91–100; Jager 1998 *Environ Toxicol Chem* 17:2080–2090). Jager (1998) pointed out that the partitioning model tends to overestimate the extent of bioaccumulation unless model calibration is allowed. However, a mechanistic model describing bioaccumulation in worm seems difficult to develop due to complications such as reduced bioavailability caused by aging/weathering and dietary/ingestive contribution to uptake. This study investigates whether it is possible to model bioaccumulation of weakly polar and non-polar organic chemicals in oligochaete beyond the partitioning model using observations reported in the oligochaete literature. In order to circumvent the problem of variable bioavailability, only bioaccumulation studies involving spiked sediments or soils were analyzed. Approximately 300 oligochaete data entries on biota-soil/sediment accumulation factors (BSAFs) and BAFs were collected and reviewed. It was found that partitioning model tended to overestimate body burden of chemicals in worm. Preliminary analysis on chemical uptake data suggested that it may be possible to differentiate uptake contributions from different uptake pathways as a function of the chemical's chemical properties. The discrepancies between the observed and the partition-based bioaccumulation estimates were calculated and compared to the extent of in vivo biotransformation predicted using a recently developed model. It was found that for highly hydrophobic compounds ($\log K_{OW} \geq 5$) the derived differences generally agreed well with the biotransformation model predictions. The results from this study suggested that it may be possible to develop a process-based mechanistic model for the prediction of bioaccumulation of organic compounds in oligochaete. Implications on risk assessment and remediation strategies are also discussed.

WE200
Bioaccumulation, Tissue distribution and Maternal Transfer of Pharmaceuticals and Personal Care Products in Zebrafish (Danio Rerio)
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Bioaccumulation, Tissue distribution and Maternal Transfer of Pharmaceuticals and Personal Care Products in Zebrafish (*Danio Rerio*)
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WE201
Validation of an HPLC method for determining log Pow values of non-ionic surfactants
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The aim of this study was to determine whether the current OECD 117 method for determining n-octanol/water partition coefficient (log P_{ow}) data for chemicals could be applied to alcohol ethoxylates (non-ionic surfactants). In order to carry out this evaluation, the log P_{ow} range of the standard calibration line had to be extended from 0-6 (OECD 117) to 0-9. This was achieved by running additional calibration standards with log P_{ow} values in the range 6-9 and also reducing the polarity of the mobile phase to achieve an acceptable HPLC run-time. Experimental studies confirmed that the components of alcohol ethoxylates elute from the HPLC column in order of their predicted hydrophobicity. The HPLC method was found to be particularly advantageous for the determination of log P_{ow} values for alcohol ethoxylate products as it can generate data for highly hydrophobic material (log P_{ow} >6) and has sufficient resolving power to separate individual alcohol components from their ethoxylated moieties. There is a strong similarity between the experimentally derived log P_{ow} values using the adapted HPLC method for these components and their predicted values using SPARC (R^2=0.89). SPARC is capable of accurately predicting the log P_{ow} values of alcohol ethoxylates with up to 5 ethoxylations, though predictions become less accurate beyond this threshold.

WE202
Evaluation of currently available methods for determining the log Kow values of surfactants
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Due to their unique properties, surfactants tend to accumulate at the interface of hydrophobic and hydrophilic surfaces, making the measurement of log K_{ow} (n-octanol-water partition coefficient) a technical challenge. The ERASM 'Hydrophobicity of Surfactants' Task Force has been assessing the suitability of existing methods for determining the log K_{ow} of surfactants. The traditional 'shake flask' method (OECD 107) is considered wholly inappropriate for the determination of log K_{ow} values of surfactants due to emulsion formation and uncertainties with regard to phase behaviour of the surfactant in the two solvents. A number of different approaches for log K_{ow} determination for surfactants (e.g. 'slow stir' method (OECD 123), HPLC method (OECD 117), computational method (referred to in OECD 107) which uses the ratios of solubilities in n-octanol and water (measured using OECD 105)and QSAR/ predictive methods have been used by the different lead registrants in REACH Phases 1 and 2. There are concerns that some of these methods have not been fully validated for surfactants and might not be applicable for such substances. The Task Force has therefore coordinated a ring test to generate comparable data using these four different methods for a select set of surfactants from the four main categories (non-ionics, anionics, cationics and amphoterics). A side-by-side comparison of log K_{ow} data for these methods will be provided. In addition, the Task Force has also been assessing a range of novel and possible alternative methods for both the measurement and modeling of hydrophobicity of surfactants. Some examples are: Liposome- water partitioning to determine $K_{lip.water}$ for soluble fractions. Determination of phospholipophilicity using Immobilised Artificial Membranes (IAM) Use of Solid Phase Microextraction (SPME) Use of various centrifugal partition chromatographic (CPC) techniques The pH metric method for ionisable substances (draft OECD 122, November 2000) \nThe pros and cons of these methods will be provided.

WE203
Development of a reference list of chemicals for evaluating alternatives to in vivo fish bioaccumulation studies
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Under the European Union REACH (Registration, Evaluation, Authorisation and restriction of Chemicals) legislation, there is an urgent need to develop and validate alternative methods to traditional *in vivo* studies to assess bioaccumulation of chemicals. The majority of *in vivo* studies have been focused on the determination of bioconcentration factor (BCF) measured in whole fish according to standardised guidelines; few studies investigating crucial processes in bioaccumulation such as metabolism have been conducted. Due to the high cost and number of animals used in *in vivo* experiments, a variety of cell-based *in vitro* assays have been developed. However, the applicability of thesemethods to assess chemical bioaccumulation is currently limited due to technical shortcomings and assay variability and lack of standardisation necessitating further improvements. The aim of this study was to develop a list of reference compounds to be used in the development and evaluation of alternative methods as a surrogate, or compliment, to *in vivo* studies for the assessment of chemical bioaccumulation. Compounds that were supported by *in vivo* data for rainbow trout (*Oncorhynchus mykiss*) were compiled from the literature and established BCF databases. *In silico* techniques were used to predict the maximal BCF value, biotransformation rate and metabolic pathways of the compiled compounds. The best candidates for *in vitro* testing included different chemical classes such as polycyclic aromatic hydrocarbons, organophosphates and a wide range of aromatic halogenated compounds. These chemicals also cover a broad range of values for key descriptors for chemical bioaccumulation: the logarithm of n-octanol/water coefficient, molecular weight and molecular diameter. A list of 114 compounds is anticipated to provide a transparent basis within the scientific community for future experimental evaluation of the applicability of alternative methods for bioaccumulation assessments with fish.

WE204
Bioaccumulation of narcotic compunds
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Narcosis follows the Fergunson's principle, in other words the rate-limiting step for narcosis is the ability of the agent to reach the site of action. The traditional lipid solubility theory of narcosis says that it is the result of an accumulation of an agent in the lipidic membranes of the cells and follows the "critical volume" doctrine. On the other hand, the "protein binding" doctrine states that narcosis is a result of the agent binding to a hydrophobic region of membrane proteins. Both theories state, as result of non polar narcosis, an altered structure and function of cell membranes. We evaluated the biological response to aromatic hydrocarbons, with Log Kow values lower than 3, have shown statistically different values between species of different trophic levels; algae being more resistant than fish and invertebrates. These differences were not significant with Log Kow higher than 3 for all organisms. Although, the CBR associated with lethal narcosis may be somewhat different for different species, much of this may be due to disparity in body size and composition rather than differences in target site concentrations. PNEC value, for these kinds of compound, obtained with one trophic level must not introduce a new uncertainty in a risk assessment protocol. The bioconcentration factor (BCF) achieved to start non polar narcosis fell almost one order of magnitude below the BCF expected for their Log Kow. Predicted critical body residues for non polar narcosis (CBRn) fell within a range, instead of a fixed value as it is established elsewhere.

WE205
A selective mobilization of PCB congeners from adipocytes during the lipolysis
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Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) that are widely spread in the marine and terrestrial biota and biomagnify throughout food chains. They tend to accumulate in lipid-rich tissues due to their highly lipophilic properties. Although the lipid tissue is considered as an internal site of storage for

PCBs, these molecules might be mobilized from the adipocytes into the bloodstream during a period of negative energy balance. This release may be problematic because it enables POPs to reach some target tissues and exert potential harmful health effects in humans and animals. Several *in vivo* studies have already followed the dynamics of PCBs released from adipose tissue during lipolysis. However, the mechanisms involved in this mobilization have been poorly studied. The complexity of the *in vivo* situation, which is characterized by a large range of POPs, does not allow understanding precisely the behaviour of individual congeners. At the moment, there is a lack of simple *in vitro* model available for the characterization of the dynamics of release of PCB congeners, differing by the number and position of chlorine atoms. In this study, we compared the kinetics of release of 3 congeners (PCB-28, -118 and -153) from *in vitro* differentiated rat adipocytes. The present experiment allowed assessing the impact of (*i*) the number and position of chlorine atoms of PCBs on their release from adipocytes and (*ii*) the presence of other PCB congeners on the mobilization rate of such molecules. Adipocytes previously contaminated with the 3 PCB congeners alone or in cocktail, underwent a lipolytic treatment with isoproterenol during 12 hours. The PCB assessment was achieved every 3 hours in cells and medium. The release of targeted PCB congeners from adipocytes was efficient during the lipolysis, with an accumulation in lipolytic medium. Interestingly, PCB-153, a hexa-CB with 2 chlorine atoms in *ortho*-position, was mobilized slower than PCB-28, a tri-CB, and PCB-118, a penta-CB, which are both characterized by 1 chlorine atom in *ortho*-position. These first results demonstrate the impact of the physico-chemical properties of POPs on their dynamics of mobilization from adipocytes. Moreover, the mobilization of PCB congeners was not influenced by the presence of other congeners within adipocytes in these experimental conditions.

WE206
Monitoring bioaccumulation of PCBs in eel; the role of ecology
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Measuring bioaccumulation of lipophilic compounds in aquatic organisms is commonly used for environmental monitoring. In The Netherlands, long term monitoring with Eel (*Anguilla anguilla*), using composite samples from 25 individual yellow eels in length class 30-40 cm, has shown that PCB and other organic contaminant levels have decreased since the eighties. Large differences in PCB levels between waters, but also between years at the same location, have been observed. It was also reported that lipid levels were decreasing in the Dutch eel and contaminants like PCBs were hypothesized to have a role in this. Recent monitoring, analysing individual yellow and silver eels, has shown that ecology of the selected species needs to be taken into account in the sampling design. In this presentation we show that a shift in sexe-ratio in the composite sample can have large effects on both the observed PCB concentration and the lipid content. Due to differences in their life cycle male yellow eels have higher lipid contents than female of the same size (30-40 cm length class), while at higher lengths females reach the same high lipid levels. As PCB concentrations are strongly correlated to lipid percentages of eel, changes in male–female ratio in the composite sample have large effects on the observed concentration. In this study It has been demonstrated that male-female ratio's are not constant in all sampled locations, nor over time. in the Dutch bioaccumulation monitoring, the observed differences in bioaccumulation of lypophilic contaminants in eels, as well as lipid levels, depend on both location and the composition of the composite sample (number of males and females). Our data show that lipid levels in yellow or silver eel are not negatively influenced by PCB levels (nor by sum-TEQ). Therefore, in bioaccumulation monitoring, ecology of the selected species needs to be taken into account in the sampling design. Otherwise bioaccumulation levels can be misinterpreted both in environmental view (comparing pollution levels between locations and over time) and in assumed eco-toxicological effects of PCB levels on lipid percentages of eel.

WE207
Bioaccumulation of Triclosan in great pond snails from a pond mesocosm study
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Triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy) phenol) is a frequently used ingredient in products of everyday such as detergents, toothpaste, cosmetics, children's toys or antibacterial textiles owing to its antibacterial properties. TCS is highly toxic to algae and various microbial species. There is some evidence of endocrine disruption. Both TCS and its metabolite methyltriclosan (MTCS) are considered bioaccumulative in aquatic organisms. While data for bioaccumulation of TCS in fish are available, empirical findings on bioaccumulation of MTCS and TCS, MTCS in other species are scarce. A combined fate and effect pond mesocosm study was carried out by the German Federal Environment Agency. After single dosing of the free water, concentrations of TCS and metabolits MTCS were measured for 120 days in aufwuchs, macrophytes, and snails (Lymnaea stagnalis). Even though conditions were not steady-state, the comparison with the measured concentrations in the water allowed for realistic calculations of the bioaccumulation factor (BAF) for both the *great pond snail* Lymnaea stagnalis and aufwuchs which is a major part of their diet.

WE208
Pollutant bioaccumulation in the invasive species Chinese Mitten Crab
S. van Leeuwen, RIKILT / RIKILT; M. van der Lee, RIKILT; M. Hoek-Nieuwenhuizen, IMARES; R. Hoogenboom, RIKILT; m. kotterman, IMARES / Fish
Chinese Mitten crab (*Eriocheir Sinensis*) is an invasive species that establishes successfully in northwest Europe in brackish waters as well as more upstream in rivers. Recent studies showed high concentration of dioxins and polychlorinated biphenyls (PCBs) in the mitten crab from the UK. In 2010-2013, several surveys were conducted in the Netherlands to profile (a) the tissue distribution of dioxins and PCBs in crab, (b) the geographical distribution in different water bodies (freshwater, brackish). In 2013, perfluoroalkyl substances (PFASs) and brominated flame retardants (BFRs) were determined to broaden the pollutant profile information. Comparisons were made with extensively monitored eel samples from the same locations. Consequences for human consumption are discussed. In all cases (except when indicated), samples concerned pooled samples, in most cases of 25 individuals. Dioxin and PCB levels are highest in the so-called brown meat (in our study defined as the hepatopancreas, gonads and other soft tissues from the crab carapace (body)). These tissues in crabs from the sedimentation areas of the Rhine and Meuse rivers were highest polluted. Levels of 12-81 pg sum-TEQ were observed. Crabs from Lake IJssel and the Wadden Sea were less polluted (8-21 pg sum-TEQ/g ww). Levels in the muscle tissue from the legs and claws (appendages) were 14-120 times lower (all except one were

WE209
Does PAH bioaccumulation by macro-invertebrates follow suspended matter pollution dynamics?
M. Frelat, CRP Henri Tudor / CRTE; r. carafa, TUODOR / CRTE; T. Galle, CRP Henri Tudor / CRTE; P. Denis, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE
Polycyclic aromatic hydrocarbons remain a source of stress in urban catchments where surface runoff and (historically) contaminated sites provide variable flows of these pollutants. As PAH are rather hydrophobic, their transport occurs dominantly in the particulate phase and is subject to the hydrological dynamics of sediment transport. Depending on the mixture of sediment sources, chemographs of particulate bound PAH show very distinct patterns in the water column during flood waves with polluted sediments appearing in early stages. The exposure of macro-invertebrates to particle-bound PAH in rivers with modest and dispersed fine sediment budgets is very difficult to assess. The patchiness of sediment deposition suggests low-flow suspended matter analysis as pragmatic alternative for exposure assessment. The PAH concentration in the low-flow samples is substantially lower than peak concentrations during flood-events. Is it important to capture the flood-wave pollution to predict bioaccumulation of PAH by macro-invertebrates? This poster contribution investigates the dynamics of bioaccumulation of insect larvae with a food-web that has been established in the model AQUATOX and uses data from autosampler campaigns (flood waves) as well as sediment nets (low-flow) to simulate PAH body residues in insect larvae. It discusses equilibrium distributions of PAH versus kinetic approaches in bioaccumulation dynamics. The influence of insect larvae traits will also be discussed with body residue validation data. In addition a new dataset of low flow suspended matter and macro-invertebrate samples from 8 sites in Luxembourg will explore the site variability of the PAH exposure.

WE210
Biotransformation of fungicides in the aquatic invertebrate Gammarus pulex
A. Rösch, Eawag / Environmental Chemistry; J. Jeon, Eawag Aquatic Research / Environmental Chemistry; J. Hollender, Eawag / Environmental Chemistry
Biotransformation describes the enzyme-catalyzed transformation of compounds by organisms. In order to facilitate excretion from the organism, the formed metabolites usually are more hydrophilic than their precursors. Thus biotransformation is a key process that can greatly influence the bioaccumulation potential and toxicity of organic compounds, leading either to detoxification or bioactivation. In the present study the strobilurin fungicides azoxystrobin, kresoxim-methyl and trifloxystrobin were chosen to study biotransformation in the aquatic invertebrate *Gammarus pulex (G. pulex).* The selected compounds exhibit a generally high toxicity to aquatic organisms by inhibiting mitochondrial respiration. *G. pulex* were exposed to the selected fungicides at concentrations of 200 $\mu g\ L^{-1}$ for 24 hours. By using high-resolution LC-tandem mass spectrometry combined with suspect and non-target screening approaches metabolites were identified. Structure elucidation was achieved by the interpretation of MS/MS spectra and with the help of MS/MS fragmentation tools (e.g. MassFrontier,

Molgen MS/MS). As expected structural similarity between the selected strobilurin fungicides lead to related biotransformation reactions. The major biotransformation pathway identified was an ester hydrolysis to its acid. Further reactions detected include hydroxylations, hydrogenations and divers conjugation reactions such as glucosidations, cysteine and sulfate conjugations.

WE212

A triple isotope (δ13C, δ15N and δ34S) approach to study bioaccumulation of legacy and current-use flame retardants in White-tailed Eagle Haliaeetus albicilla

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Stable isotopes are increasingly employed to investigate how dietary specialisation, e.g. food chain origin (δ^{13}C) and trophic level (δ^{15}N), may influence bioaccumulation. In this regard, the usefulness of sulphur stable isotope (δ^{34}S) is still poorly investigated, although recent studies have shown their potential as dietary tracer. We employed a triple-isotope approach (δ^{13}C, δ^{15}N and δ^{34}S) to investigate bioaccumulation of organochlorines (OCs), brominated flame retardants (BFRs) and phosphorus flame retardants (PFRs) in White-tailed Eagle *Haliaeetus albicilla* nestlings. Nestlings are especially sensitive to toxic effects from pollutants and enable to study small-scale spatial variation in exposure and dietary habits. In addition, sampling at the nestling stage minimises possible confounding by intrinsic factors. Stable isotopes and pollutant concentrations were both analysed in body feathers. PFR concentrations (0.95-3,000 ng/g) were much higher than all OCs (2.3-21 ng/g), PBDEs (0.03-2.3 ng/g) and non-PBDE BFRs (0.03-1.5 ng/g). A model selection procedure, based upon Akaike Information Criteria (AIC), showed that most OCs and PBDEs, as well as tris(chloroethyl) phosphate (TCEP), tris(phenyl) phosphate (TPHP) and tri-(2-butoxyethyl) phosphate (TBOEP) weresignificantlyassociatedto δ^{15}N, δ^{13}C, or a combination of both (all $P \leq 0.02$). The frequent recurrence of δ^{15}N in the most parsimonious models showed that bioaccumulation was indeed a prevailing process. δ^{34}S was at times identified as an important factor as well, though never reached significance ($P \geq 0.07$). Innestlings sampled close to a city,δ^{15}N and δ^{13}C were both significantly enriched, whileδ^{34}S was significantly depleted. Enriched δ^{15}N and δ^{13}C, conventionally suggesting that these individuals were fed more with a marine diet, contradicted depleted δ^{34}S suggesting a more terrestrial diet. As such, these results indicated more likely that agricultural and industrial human activities associated with the city may have influenced δ^{15}N and δ^{34}S, respectively. Therefore, both δ^{15}N and δ^{34}S may serve as a spatial indicator for point sources. Problematically though, this new perspective on δ^{15}N may confound its established use as a proxy for trophic level.

WE213

Evaluation of Polycyclic aromatic hydrocarbons (PAHs) contamination in estuarine ecosystem in Brazil

J. RIZZI; S. Froehner, Federal University of Paraná / Department of Environmental Engineering

Estuarine environments are very delicate systems that provide habitat for a large number of organisms. However it's also a final destination of many chemical compounds due to economic, industrial, touristic and agricultural activities. Usually, the main cause of deep perturbation on estuarine are the unorganized and unplanned occupations also, port activities contributes to degradation The presence of organic toxic compounds released into the environment has grown exponentially and most of them indeed are from anthropogenic sources. One of the concerning about the presence of organic compounds in sediments and water is the biomagnification. Biomagnification can be defined as the process in which an organic compound in concentrated higher up along the food chain. However, it can be difficult to identified organic compounds in lower level of food chain. In this work we scanned the presence of the 16 priority polycyclic aromatic compounds (PAHs) in water and sediments of Paranagua Bay, in South of Brazil.: This region has been under pressure due to urban occupation and also by expansion area and increment of port and dredging activities. It had reported the presence of PAHs in surface sediments, due accidental oil spills. Not only sediment and water samples were collected, but also plankton and fish completed the monitoring. The purpose was to assess the potential of such compounds to spread from sediment and water to ecosystem. A mathematical model was applied to understand the fate of PAHs compounds considering the trophic web. Finally, the analytical results are close to the results predicted by mathematical model. The results showed that the compounds can be found beyond the sediments. Further ecotoxicological analyses are required to complete understand the consequences of this contamination on ecosystem.

WE214

Binding of tributyltin or tetrodotoxin to recombinant saxitoxin and tetrodotoxin binding proteins from the pufferfish, Takifugu rubupires

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Tributyltin (TBT) is a well-known environmental pollutant in aquatic ecosystems. High concentrations of TBT have been detected in the blood of 13 species of fish, including pufferfish (*Takifugu rubupires*), collected off the coast of northern Kyushu, Japan (Miki et al., 2011). This high accumulation might be caused by the presence of TBT-binding proteins (TBT-bps) in the blood of these fish (Shimasaki et al., 2002). Previously, we demonstrated that pufferfish saxitoxin and tetrodotoxin (TTX) binding proteins (PSTBPs) have duplicated amino acid sequences of TBT-bps (Satone et al., 2010). Thus, we suspected that the accumulation of TTX and TBT in blood of pufferfish may be caused by their binding to PSTBPs in the blood. However, the affinity of PSTBPs to TTX and TBT are currently unknown. To investigate these affinities, we used a silk worm baculovirus expression system to purify recombinant PSTBP1 and PSTBP2 from *T. rubupires*. Using an ultrafiltration and competition assay, we showed that while recombinant PSTBP2 can bind to both TTX and TBT, recombinant PSTBP1 can only bind to TBT. As resuls, PSTBP migh be evoluved from TBT-bps,maintaining its detoxification function.

WE215

DEGRADATION OF METHYL AND PROPYL PARABEN IN LABORATORY TEST WITH NILE TILAPIAS (OREOCHROMIS NILOTICUS)

D.C. da Silva, Chemistry; C.A. Galinaro, Universidade de Sao Paulo / Chemistry; L.R. Diniz, São Paulo State University Institute of Chemistry; L.A. Capellini, Universidade Sao Paulo / Departamento de Quimica e Fisica Molecular; L.A. Serrano, Universidade Sao Paulo / Departamento de QuímicaFísica Facultad de Ciencias del Mar y Ambientales; E.A. Almeida, Chemistry and Environmental Sciences; E.M. Vieira, Sao Paulo University / Departamento de Quimica e Fisica Molecular

DEGRADATION OF METHYL AND PROPYL PARABEN IN LABORATORY TEST WITH NILE TILAPIAS (OREOCHROMIS NILOTICUS) Daniele Caetano da Silva[1]*, Carlos Alexandre Galinaro[1], Lia Gracy Rocha Diniz[1], Luciana Teresa Dias Capellini[1], Lenard Alexandre Serrano[1], Eduardo Alves de Almeida[2], Eny Maria Vieira[1]. [1] Universidade de São Paulo, Instituto de Química de São Carlos, São Carlos, São Paulo; [2] Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas, São José do Rio Preto, São Paulo. * Contact: dani_caetanods@hotmail.com Parabens are alkyl esters derived by p-hydroxybenzoic acid used alone or in combination with other compounds, as preservatives in cosmetics, foodstuffs and pharmaceuticals. However, many of these compounds that are released into bodies of water and can cause damage to organisms that are continually exposed multixenobiotic. Furthermore, in the sewage treatment stations there is no specific method to eliminate them. Thus, there are few studies focused on the performance of these preservatives in the aquatic environment over time. This study tried to clarify the hypothesis that if after 6 days, methyl and propyl paraben will be absorbed by Nile tilapia or degraded and what would be its action during this period. Two tests were performed with mixture (methyl and propyl paraben), one in the presence of one fish and other without. Each experimental group had 6 animals and each fish had its own aquarium with 20L each. The concentrations of the compounds were determined by high performance liquid chromatography with diode array detector (HPLC-DAD) from laboratory tests. Was observed a gradual decrease in the concentration of the compounds during the 6 days of testing. In the group with mixture and fish, after 2 days, we did not find any of the compounds in water. In the mixture Group, there was a decrease in concentrations after 3 days. We observed that only the methylparaben was degraded completely, while the concentration of propyl was decreased gradually. These results, demonstrating the rapid uptake of the compounds by aquatic organisms, may bioaccumulate in different tissues, Furthermore, we observed that the more toxic is the compound, the more persistent is in the environment. (FAPESP 2012/00150-0). Keywords: methhylparaben, propylparaben, Nile tilapia.

WE216

Effect of calcium and nutrients on strontium-90 uptake and distribution for dose rate assessments in Lemna minor

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The potential releases of different radioactive elements into the environment can have a biological impact on living organisms. For a number of radionuclides there is still a lack of understanding on the way they are taken up and internally distributed

in non-human biota, and plants in particular. Among radio-ecologically relevant radionuclides, radioactive Sr^{90} is one of the most common radioisotopes originating from nuclear activities and accidents and has a relative long half-life of 29 years. Although this pure β-emitting radionuclide is not essential for plant metabolism, it bears a chemical analogy with the essential plant macronutrient Ca thereby taking advantage of Ca transport systems to accumulate into plant organs and tissues. Ones plants are contaminated with Sr^{90}, ionization events of this radioactive isotope typically can cause biological damage. The main objective of this work was to examine Sr^{90} concentration factors for various medium conditions in a 7-day time scale for root and frond. The macrophyte *Lemna minor* was chosen as model system. Different standard media were tested on growth rate and biomass to analyse the amount of Sr^{90} taken up by the plants exposed to 5 or to 15 kBq/L. Furthermore, Sr^{90}-uptake was also measured as a function of time over a 7-day incubation period for fronds and roots separated. These data were directly applied in dose rate assessments for the investigation of β-radiation stress in the plants. It turned out that the radionuclide was taken up to a level that was proportionally related with that of external Ca concentration. However, these plants showed various morphologies and densities among selected medium. Also, the plant equilibrated rapidly with the Sr^{90} concentration in the growth medium with an equal distribution between root and leave structures. These data are important to implement a dynamic dosimetric model for the determination of internal and external exposures rates with respect to variable size, density and morphology of aquatic plants. The total dose rates corresponded well at increasing activity concentrations of the growth medium and a significant reduction on root size was observed at dose rate of 500 kBq/L. This approach allows a high-level dose rate assessment to evaluate β-radiation stress responses in higher plants.

WE217

Relating subcellular copper partitioning to the growth effect in Ruditapes philippinarum (Bivalvia: Veneridae) exposed to continuous, increasing and pulse impact of copper contaminated sediments

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It is now well recognized that metals bind to a range of biological ligands at sites that have different functions. They can be detoxified or excreted, but only the metabolically active fraction of metal contributes to toxicity. The present study investigates the links between the subcellular metal exposure and toxic effect to growth of the bivalve *Ruditapes philippinarum* exposed to increasing, continuous and pulse impact of copper-spiked sediment. Estuarine sediment from a pristine site in the Bay of Cadiz (SW Iberian Peninsula; 36º23'31.80''N, 6º12'24.01''W) was collected and characterized for grain size (25% particles < 63µm), particulate organic carbon (2% POC) and metal content (28 µgCu/g). Sediments were spiked in order to yield three increasing copper concentration series (8, 12 and 20 mg Cu < 63µm /g POC) and allowed to equilibrate for 30 days, stored at 4°C. Experiments were conducted in triplicate during 40 days exposing juvenilesof *R. philippinarum* (acclimated 7 days in laboratory controlled conditions) to the contaminated sediments in a flow-through system. Four experiments were set up: Control 1) a control with unspiked sediment; Experiment 1) where organisms were subsequently exposed to increasing copper concentration series during 10 days each and then allowed to recover in control sediment 10 days; Control 2) where organisms were maintained at the highest concentration series for 30 days and then changed to control sediment and Experiment 2) where bivalves were exposed to a pulse impact alternating the highest copper concentration series and the control every 10 days. Each 10 days, 10 clams were sampled, depurated 24h in clean seawater, weighed and the soft tissues were frozen at -80°C until analysis. Throughout the experiments subsamples of sediment, pore water and overlying water were analyzed to determine copper concentration and physico-chemical variables (pH, DO, salinity and temperature) were recorded. Total copper-residue concentration and subcellular fractionations were determined to investigate the linkage of subcellular partitioning with species' mechanisms of growth toxicity. Understanding both metal exposure and subcellular partitioning of the metal within an organism may considerably improve our ability to predict the toxicity of metals.

WE218

Influence of sediment modifying factors on uranium bioaccumulation in the freshwater midge

S.E. Crawford, University of Saskatchewan Toxicology Centre / Toxicology Centre; K. Liber, University of Saskatchewan / Toxicology Centre

Sediments act as a reservoir for many metals; however, our knowledge and understanding of how physicochemical characteristics of sediment alter the toxicity and bioavailability of some metals is incomplete. In particular, there is limited information regarding the bioavailability and toxicity of sediment-associated uranium (U). Thus, the goal of this research is to quantify sediment characteristics that influence bioavailability and hence toxicity of sediment-associated U to a model freshwater benthic invertebrate. The freshwater midge, *Chironomus dilutus*, were exposed to both field-collected and formulated sediment spiked with U for 10 d to determine differences in U bioaccumulation from sediment with varying physicochemical properties. The physicochemical characteristics of sediment that were examined in this research include particle size distribution and organic matter (OM) content, as they are predicted to alter the partitioning and bioavailability of U and other metals associated with sediment. Formulated sediments were prepared to mimic the physical and chemical properties of field-collected sediment in order to quantify the influence of different sediment physicochemical characteristics on U bioavailability without test artefacts associated with field sediment. Tests were conducted with control sediment and sediment spiked with sub-lethal U concentrations of 5 to 200 mg U/kg dry weight (d.w.) in formulated or field sediments, aged for 20 d. Test endpoints and measurements included midge survival and growth, and U concentrations in whole organisms, whole-sediment and water (both overlying water and pore water). In all 10-d experiments, *C. dilutus* survival was above 80% regardless of sediment type, U concentrations, or physicochemical characteristics. Formulated sediments prepared to resemble U-spiked field collected sediment resulted in statistically similar U bioaccumulation in exposed test organisms, suggesting formulated sediment is a suitable alternative to field sediment with respect to U bioavailability. Additionally, field and formulated sediment spiked with U resulted in an inversely proportional relationship between increasing concentrations of clay or OM and decreasing U bioaccumulation in exposed organisms. This research demonstrates that physicochemical characteristics of sediment, such as particle size and OM, play an important role in modifying U bioavailability associated with sediment and could be used to improve risk assessments of contaminated sediment.

WE219

Metal bioaccumulation in natural river biofilms collected in an urban contamination gradient

J. Fabure, M. Dufour, Irstea / UR HBAN; L. Fechner, UR HBAN

Metal bioaccumulation in river biofilms is important to monitor metal contamination of aquatic systems and also an interesting alternative to the use of experimental animals for biota contamination assessments. However, bioaccumulation in biofilms is a complex process and the links between exposure and biofilm-accumulated concentrations need to be investigated. The aim of this study is to investigate the seasonal variability of metal bioaccumulation of periphytic communities (river biofilms) chronically exposed to a multi-metallic pressure. Biofilms were grown *in situ* on immersed plastic membranes at three sites on the Seine river along an urban pollution gradient upstream (site 1) and downstream (sites 2 and 3) from Paris (France). Four sampling dates were chosen at different seasons (September 2011, March 2012, July 2012 and December 2012) and biofilms were collected after a 3 to 5 weeks colonization period. Metal (Ag, Cd, Cr, Co, Cu, Mn, Ni, Pb, Zn, Sb) accumulation within the biofilms (intracellular and total accumulated metals) was measured (with and without prior EDTA washing respectively) after acidic mineralisation of the samples. Total, dissolved, and DGT-labile (with Diffusive Gradient in Thin films) metallic concentrations were monitored as well as major physico-chemical parameters in the river water at all sampling dates and sites. Concentrations of metals accumulated within the biofilms reflected the increase of the multi-metallic exposure along the urban gradient as they were always lower for the site 1 biofilm, except in the case of Ni and Cr where accumulated levels were similar at all sites for some sampling dates. The highest contamination levels were observed in the winter biofilms (sampled in December 2012) collected at sites 2 and 3 (downstream from Paris). Biofilm-accumulated concentrations had a particularly high correlation with water contamination levels for metals like Cu ($R = 0.70$ with total Cu), Pb ($R = 0.70$ with total Pb) and Zn ($R = 0.62$ with dissolved Zn and 0.47 with total Zn). Seasonal variations of biofilm-accumulated contamination levels were further explored using multivariate analysis. This study shows that chronic *in situ* exposure to low, environmental concentrations of metals has a significant impact on biofilm metal bioaccumulation and reveals the importance of exploring the links between metal speciation and metal bioaccumulation in biofilms.

WE220

Copper, cadmium and zinc exposures in the coral prawn, Metapenaeopsis crassissima: Males Vs. females and the role of cadmium metallothioneins

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The coral prawn, *Metapenaeopsis crassissima*, a marine species from Shark Bay, Western Australia is unique amongst crustaceans because firstly, the female has significantly higher concentrations of cadmium (Cd) than the male and secondly this Cd is located in their tail muscle, a tissue that usually has one of the lowest Cd concentrations in crustaceans. This increased Cd is associated with the presence of several Cd metallothioneins (CdMT) isoforms The aim of these experiments was to examine the impact of Cu exposure, in the presence of either Cd or Zn, on metal accumulation rates, and accompanying MT induction, in the tail muscle of male and female *M. crassissima*. Male coral prawns exposed to CuZn had earlier mortalities, concomitant with an increased mortality rate, compared with the females and to

both males and females exposed to CdCu. The Cu and Zn uptake rates in the muscle, for those prawns exposed to CuZn, were significantly greater in the male than the female prawns. This contrasts to the CdCu exposure where there was no difference between males and females. Cadmium MTs, were present in those prawns exposed to CdCu with a stronger induction, including more isoforms present, in the female than the male. However, there were minimal CdMTs detected in the tail muscle of the female prawns after 48 hours exposure to CuZn and no CdMTs detected in the males. The presence of at least six isoforms of CdMTs in the female prawn tail muscle appear to provide a short term protective effect against the toxicity of Cu which is not observed in the males. The Cu appears to initially replace the Cd ions in the CdMTs present in the tail muscle, and once saturation occurs the mortality pattern of Cu toxicity in females reflects that of the males, albeit delayed.

WE221
Regulation of body metal concentrations: toxicokinetics of Cd and Zn in crickets
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Several studies have shown that internal body concentrations of nutritional metals (eg., Zn, Cu) are regulated efficiently by invertebrates, whereas other metals (e.g., Pb, Cd) are not. However, it is not known whether this regulation is achieved through decreasing assimilation rate (*ka*), increasing elimination rate (*ke*), or both, and whether all metals are regulated in a similar manner, even if with different efficiency. The regulation of metal concentrations in invertebrates, at least when a metal enters the body with food, can be simply the side-effect of metal toxicity to gut epithelial cells: the higher the metal concentration in food - the higher the damage to the gut. If this is the case, it should be reflected in decreasing assimilation efficiency and/or increasing elimination rate (mostly thanks to shedding damaged epithelial cells) of metals with increasing metal concentration in food. The magnitude of these effects should also depend on the inherent metal toxicity. In this study, the crickets *Gryllus assimilis* were exposed to Zn or Cd at three different concentrations (2.5, 10, and 40 mM/kg dry food), and toxicokinetics of metals were followed for four weeks. The same molar concentrations of both metals were used to be sure that animals are exposed to the same amount of metal ions. The difference in inherent toxicity of the metals was seen in the significant increase of cricket mortality with increasing Cd concentration, while no such effect was observed in Zn-treated animals. In case of Zn a clear increase in body concentration was found only at the highest treatment, while at the lowest the internal concentration remained unchanged throughout the whole experiment. At the lowest Zn concentration, the estimated *ka* and *ke* perfectly balanced each other (*ka*=0.024, *ke*=0.024). With increasing Zn concentration in food, *ka* decreased to 0.02 at 10 mM/kg and 0.01 at 40 mM/kg, and *ke* increased to 0.05 and 0.07, respectively. It seems, thus, that body concentration of Zn is regulated by changing simultaneously *ka* and *ke*. In contrast, even at the lowest treatment a significant Cd concentration increase was observed in crickets. Cd concentration was regulated almost exclusively through increasing *ke*: from 0.14 at 2.5 mM/kg, through 0.36 at 10 mM/kg to 0.61 at 40 mM/kg. The project was financed by the Polish Ministry of Science and Higher Education (Project No. NN304038440).

WE222
Selenium Bioaccumulation: Impact on the Fat Body Histology of Silkworm Bombyx. Mori L.
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In the present investigation, an attempt has been made to study the histological aspects fat body of *Bombyx mori L.* and changes under exposure to selenium. The insect fat body is the principal organ of intermediary metabolism site for the requirement of the physiological activity and is similar to the liver of mammals in function. Fat body accumulates selenium and the bioaccumulation increased with increase in Selenium dose and period of exposure. The rate of bioaccumulation increased with period of exposure in silkworms, which received the sub-lethal dose of selenium and was significantly less when compared to the silkworm treated with lethal dose of selenium. Microscopic study of fat body revealed interesting pathological changes in all the lethal dose of selenium treated silkworms. The changes in tissues are dose and time dependent. In sub lethal dose of selenium treated silkworms, mild to severe changes were observed on the 3 day of exposure. Whereas very mild and insignificant changes were observed in the sub lethal selenium treated silkworm on further exposures at 4, 5 and 6 days. In conclusion, selenium in fat body exhibited a persistent relationship between the histopathological changes and the levels of selenium.

WE223
Seasonal variability of metal bioaccumulation in gammarids and inter-species comparison: a biomonitoring investigation under an urban diffusive contamination gradient
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Because of their functional roles in aquatic ecosystems and their wide distribution in Europe, the invertebrates of the genus *Gammarus* are candidates commonly used as tools for biomonitoring of the bioavailable fraction of metals in freshwaters. However, the interpretation of bioaccumulation data can be hampered by climatic factors. Indeed, the seasons can influence the physiological processes of animals including bioaccumulation. Furthermore, the ability of these amphipods to regulate metals can vary one species to another. To date, the genericity of the responsiveness of gammarid species to metals remains to be assessed. In order to assess the relevance of using gammarids as biomonitors, we investigated the seasonal influence on metal bioaccumulation between two species of gammarids, *Gammarus pulex* and *G. fossarum*, exposed to a multi-metal contamination under field conditions. Calibrated gammarids were collected in two freshwaters not impacted by metals and from two different river basins (*G. fossarum*: Rhone basin and *G. pulex*: Seine basin). Then, gammarids were encaged and deployed on 3 sites of the Seine, i.e. a site upstream and two sites downstream of the megacity Paris, in autumn, winter, spring and summer (2011-2012). After a week of exposure, metal concentrations were determined in animals, i.e. Ag, Cd, Cr, Co, Cu, Mn, Pb, Ni and Zn. Total, particulate, dissolved and labile metals were also monitored during the exposure and the main physicochemical parameters of water were determined on each site. Results show that contamination patterns of gammarids are similar between two species, and closely linked to the contamination gradient of the Seine river. Statistical analyses indicated that only Ni bioaccumulation in two gammarid species is influenced by the seasons, especially by the water's temperature. For all other metals, the variation in contamination levels of sites during seasons was the major explanatory factor of the metal concentrations in gammarids. Finally, the bioaccumulation factors (BAFs) were not significantly different between the two species, suggesting similar abilities to regulate internalized metals. This study supports the assumptions that gammarids from control populations of different geographical origins, have the same responsiveness to metals and, that their use should provide a reliable measure of metal bioavailability in freshwaters whatever the season.

WE224
Heavy metals in shells: considerations around a possible bioinertization process by using bioaccumulation factors
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It's well known that Bivalves are filter feeders and consequently efficient accumulators of contaminants and ideal indicator organisms: this is the reason why they are widely used in biomonitoring programs to assess pollutants levels in marine and transitional environments. In most cases experiments are conducted to evaluate concentration levels in soft tissues: in this case we want to focus the attention on shells composition, in order to better understand mechanisms that control inorganic contaminants transfer to clams carbonatic parts. We examined five sites located in the Venice lagoon: in particular we measured heavy metals levels in sediments, in *Venerupis philippinarum* soft tissues and shells and then we evaluated bioaccumulation trends. The ability of the shell carbonate crystalline structure to include elements different from calcium such as metals at levels depending on environmental concentrations is recognized by the scientific community. But there is still no agreement if this kind of process can be considered a bioaccumulation process. So we analyzed the bioaccumulation factors resulting from the comparison of sediment concentration with heavy metals concentrations in the shell carbonate matrix and not only with soft tissues ones. The study provided useful information to characterize the shell production process operated by Bivalves such as a heavy metals bio-inerting phenomenon or at least such as a pollutants bioaccumulation process in the marine environment. For the description of bioaccumulation trends we used bioaccumulation factor (BAF) relating the total heavy metals concentration in the environmental matrix (sediment) with the concentration in Bivalve tissues and shells: we obtained a number expressing metals contents in organisms as a results, on the one hand, of Bivalve specific uptake paths (metal bioaccessibility) and, on the other hand, of pollutants bioavailability in sediments.

WE225
Modelling Pb and Cd bioaccumulation in Gammarus pulex: Application to realistic environmental conditions and importance of water chemistry
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Bioaccumulation is a good indicator of the metal exposure in of aquatic organisms and also enables to integrate the effect of water chemistry on metal bioavailability, fraction expected to be toxic for biota. However, the link between water's

contamination and metal concentration in organisms is complex. Indeed, bioaccumulation depends on various physicochemical parameters, such as water cationic composition which can influence metal uptake by competitive binding on biological surfaces. The development of bioaccumulation models as a tool for quantifying metal bioavailability constitutes promising approaches for understanding and predicting metal impacts on aquatic ecosystems. The present study aims at evaluating the suitability of using models calibrated in the test organism *Gammarus pulex* to predict the bioaccumulation of Pb and Cd in field conditions. In laboratory, gammarids were exposed to dissolved Pb or Cd in order to determine the uptake and elimination rate constants (k_u and k_e) for each "metal/*G. pulex*" couple which are required to model bioaccumulation. Then, gammarids were exposed to constant concentrations of metal under various environmentally realistic concentrations of major ions (Ca^{2+}, Mg^{2+} and Na^{+}) in order to evaluate theirs effects on k_u and k_e. Finally, model suitability was assessed through the comparison between model prediction and measured bioaccumulation from gammarids transplanted on French watersheds all different in terms of contamination and water chemistry. Experiments show that only Ca^{2+} has a significant competitive effect on Pb and Cd uptake which was integrated into models thanks to the expression of k_u as a function of Ca^{2+} levels and a metal-dependent affinity constant $Ki(Ca^{2+})$. Then, comparison between predicted and measured bioaccumulation from gammarids transplanted into the field reveals that both Pb and Cd models accurately predicted bioaccumulation. This suggests that k_u and k_e determined in laboratory are applicable to the conditions encountered in environments. Furthermore, the consideration of Ca^{2+} influence into the models had permitted to improve the predictions for Pb and Cd. These results highlight the significance to consider the effect of water chemistry on metal bioavailability to understand metal bioaccumulation under field conditions. So, bioaccumulation models are potentially suitable to monitor freshwater quality and to improve the assessment of the impact of metal contamination on aquatic ecosystems.

WE226
Chlordecone decontamination potential in growing male goats
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The former use of chlordecone (CLD) in the French West Indies to fight against the banana black weevil has resulted in long-term pollution of soils. In polluted areas, CLD is known to be transferred into meat of animals reared outdoors. Therefore, decontamination studies of CLD contaminated animals are of real interest for safety of local populations. In this study, 16 two month old weaned kids were submitted either to a control diet based on hay and concentrate (group 1) or to a high energy diet (control diet + corn, group 2) in order to achieve a higher daily energy intake (+66%) and to increase the adipose tissue of the animals (group 2). The animals of both groups were intravenously administered a CLD dose (1mg/kg body weight) in three successive CLD injections (CLD powder dissolved in Cremophor EL) to ensure a uniform distribution of CLD in the organism. Two days after the last intravenous injection (d0), blood samples were collected and four animals of each group were slaughtered in order to collect the liver, the peri-renal fat and the complete carcass. The 4 remaining animals of each group were reared for an additional 30 d period (d30) in order to evaluate the decontamination process. These animals were also slaughtered and the same samples were taken. All samples were subjected to CLD analysis. At d0, CLD was detected at high levels in all samples (group 1, serum: 886±126 ng/g, liver: 14718+-1453μg/kg, peri-renal fat: 601+-13μg/kg, whole carcass: 1530+-18 μg/kg) (group 2, serum: 520+-105 ng/g, liver: 13898+-867μg/kg, peri-renal fat: 711+-88μg/kg, whole carcass: 1363+-106μg/kg). CLD concentrations were found in a same range in animals of both groups with a highest concentration in liver. At the end of the experiment, CLD concentrations were significantly lower in all matrices (group 1, serum: 152+-13ng/g, liver: 2133+-428μg/kg, peri-renal fat: 132±41μg/kg, whole carcass: 198±38μg/kg) (group 2, serum: 168±36ng/g, liver: 2500±634μg/kg, peri-renal fat: 152±27μg/kg, whole carcass: 231±73μg/kg). Thus, in a short period of 30 d, the total CLD amount was found to decrease by about 80% in both control and high energy fed animals. CLD was rapidly excreted from the animal organism and did not appear to be strongly linked to adipose tissue. Such results give interesting inside in terms of decontamination and safety of animal products.

Predicting molecular properties of environmental contaminants (P)

WE227
Investigating Chemical Biodegradation with Artificial Neural Network Ensembles
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A chemical's biodegradation rate is an important property because compounds that persist longer in our environment are potentially more hazardous than those that readily biodegrade. However, experimental measurement of chemical biodegradation is expensive and takes up to a month to perform. Thus, computational methods to predict biodegradability are desirable. This work focuses on several important factors relevant to predicting chemical biodegradation and understanding the reasons for those predictions. First, experimental measurements for ~1600 compounds assayed using either the Japanese Ministry of International Trade and Industry (MITI) or MITI-I (Organization for Economic Co-operation and Development (OECD) test guideline 301C) protocol identified by Cheng et al.[1] were obtained. Second, structural classes which increase or decrease the likelihood of biodegradation were explored. Third, matched molecular pair analysis was used to identify pairs of similar compounds having drastically different biological oxygen demand (BOD) values. Fourth, an artificial neural network ensemble (ANNE) model was developed and validated. Descriptor Sensitivity Analysis (DSA) indicated that molecular hardness and electronegativity are important descriptors for predicting chemical biodegradability. Finally, compounds predicted to have low water solubility were investigated as a potential source of experimental false negatives. [1]Cheng, F., *et al.* J. Chem. Inf. Model **2012**, 52(3), 655-669.

WE228
Mapping partitioning properties and bioaccumulation potential onto GC×GC chromatograms of complex mixtures containing nonpolar analytes
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Environmental partitioning properties control bioavailability and transport of organic compounds in natural and engineered systems. However, reliable property data is often not available for the hundreds to thousands of potentially bioaccumulative contaminants found in complex mixture of nonpolar analytes. In the present study, we showed that these partitioning properties can be mapped onto the separation space offered by comprehensive two-dimensional gas chromatography (GC×GC). We used GC×GC chromatogram retention information to estimate environmental partitioning properties for a diverse set of non-polar halogenated and hydrocarbon compounds that undergo negligible or limited hydrogen bonding interactions. Partition coefficients relevant to bioavailability and bioaccumulation such as those for air-lipid, air-passive sampling polymer, air-water, octanol-water, water-passive sampling polymer, water-biomembrane, water-lipid, water-protein, water-dissolved organic matter, and water-sediment organic carbon partitioning systems were fitted with root mean square errors ranging from 0.15 to 0.50 log units. Property maps overlaid onto GC×GC chromatograms allow the modeling of bioavailability, bioaccumulation, and inter-phase mass transfers of large number of resolved nonpolar analytes found in complex mixtures. Thus, GC×GC can be used as a powerful tool for the risk assessment and fate modeling for complex mixtures of nonpolar organic compounds in environmental media. **Keywords:** comprehensive two dimensional gas chromatography,environmental partitioning property, fate model, bioavailability, bioaccumulation

WE229
Calculation of physicochemical properties for Short- and Medium-Chain Chlorinated Paraffin
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Chlorinated paraffins (CPs) are a complex mixture of polychlorinated n-alkanes of the molecular formula $C_nH_{2n+2-x}Cl_x$ that have carbon chain lengths from10 to 38 carbon atoms and a chlorine content of 30%–70% by weight. They are classified according to their chain length into short-chain CPs (C_{10}–C_{13}), medium-chain CPs (C_{14}–C_{17}), and long-chain CPs (C_{17}–C_{30}). Short- and medium-chain chlorinated paraffins are potential PBT chemicals (persistent, bioaccumulative, toxic) and short-chain chlorinated paraffins are under review for inclusion in the UNEP Stockholm Convention on Persistent Organic Pollutants. Despite their high production volume of more than one million metric tonnes per year, only few data on their physicochemical properties are available. Chemical property data are, however, important to study the distribution and fate of chemicals in the environment. We calculated subcooled-liquid vapor pressure, subcooled-liquid solubility in water and octanol, Henry's law constant for water and octanol, as well as the octanol-water partition coefficient with three independent property calculation methods: COSMOtherm, SPARC, and EPI Suite™ and compared our calculations to experimental data from the literature. For all properties, best agreement between calculated and measured data was obtained for COSMOtherm; results from SPARC were in good agreement with the measured data except for subcooled-liquid water solubility, whereas EPI Suite™ showed the largest discrepancies for all properties. After critical evaluation of the three property calculation methods, a final set of consistent property data for short- and

medium-chain chlorinated paraffins was compiled. Analysing the physicochemical properties of the CPs, we noticed that CPs show, for some properties, a different influence of the chlorine content compared to aromatic organochlorines, such as polychlorinated biphenyls (PCBs). Different trends were observed for the subcooled-liquid solubility in water and the octanol-water partition coefficient. Both showed no dependence on the chlorine content until 55% chlorine for CPs, while the subcooled-liquid solubility in water strongly decreased and the octanol-water partition coefficient strongly increased with increasing chlorine content for PCBs. The data set we present can be used in further studies to assess the environmental fate and human exposure of this relevant compound class.

WE230

Influence of 3D molecular structure on partitioning and sorption behaviour of organic chemicals – α cyclodextrin binding as a test case

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Protein binding is controlled by the three dimensional structure of the ligands and the position of functional groups. Sorption processes to micro porous materials such as zeolites are also influenced by the 3D structure. For accurate prediction of such binding and sorption behaviour, the underlying sorption and binding mechanisms should be understood; therefore the influence of the 3D structure on the binding was investigated with α cyclodextrin (CD) as a model system, because here the shape constraints of the model system are well defined and rather easy to understand. Thus, it may serve as a useful model material to study 3D effects that may occur in a similar way in protein binding. It has been shown that the binding of many different chemicals to CDs has frequently been interpreted as host-guest complexes. But there is no systematic investigation about this complex formation for a diverse set of chemicals. Such a study is needed for the development of a general applicable model. In this work, passive sampling and head space methods were used to determine the equilibrium partitioning coefficients between water and αCD for different organic chemicals. Based on the results for the partitioning coefficients possible sorption mechanisms are discussed which consider the different types of functional groups and the 3D structure of molecules. The results show that all studied chemicals with linear alkyl chains form stable host-guest complexes. Even long chain chemicals have high partition coefficients which suggest that all CH_2 groups fit in the CD cavity. The partition coefficient depends also on the position of the functional group; chemicals with the polar functional group at the end show higher sorption than those with the functional group in the middle of the alkyl chain. This leads to the hypothesis that the polar functional group prefers to interact with the surrounding water molecules instead of the CD cavity. The dataset was used for several QSAR approaches provided by open3DQSAR. Two different approaches were used to align test chemicals in the 3D space, an unsupervised method and a method that uses chemicals with high partitioning coefficients as a template. The performance was compared to a poly parameter linear free energy relationship model. In addition, 3D descriptors generated by COMSOsar3D were also used to model the partition coefficients. The results of this work give valuable implications in the formation of host-guest complexes of αCD.

WE231

UFZ-LSER Database: Descriptors and Parameters for Linear Solvation Energy Relationships (LSERs) and Polyparameter Linear Free Energy Relationships (PP-LFERs)

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Linear solvation energy relationships (LSERs), or more generally, polyparameter linear free energy relationships (PP-LFERs) have been increasingly used in environmental chemistry to estimate equilibrium partition coefficients of organic contaminants. LSERs and PP-LFERs are multiple linear regression models that use several solute molecular properties as descriptors. Because of the mechanistically-based modeling concept, these models represent the most robust prediction methods available to estimate partition coefficients. Solute descriptors needed for LSERs and PP-LFERs have been published for several thousands of compounds in the literature. More and more solute descriptors are determined for environmentally relevant chemicals such as brominated flame retardants, pesticides, and organosilicon compounds. However, these literature data exist in a highly scattered manner, which appears to prevent their widespread use. To improve the accessibility of such literature data, we have established the "UFZ-LSER Database" (http://www.ufz.de/index.php?en=31698). This database contains experimentally determined solute descriptors, namely *E* (excess molar refraction), *S* (polarizability/dipolarity), *A* (solute hydrogen bond acidity), B/B^0 (solute hydrogen bond basicity), and *L* (logarithmic gas-hexadecane partition coefficient) for ca 3000 compounds. The database also includes the McGowan's molar volume (*V*) calculated from the molecular structure. A list of the parameters that describe the properties of partitioning systems is also provided. The database is freely accessible for any user via the web-interface (at the URL shown above) that offers the possibility to search descriptors based on the compound name, CAS number and canonical SMILES. The solute descriptors and system parameters in the database allow the estimation of a myriad of partition coefficients of environmentally relevant chemicals for environmental, engineered, and biological systems.

WE232

iSafeRat® HA-QSARs vs. commonly used predictive models : A statistical comparison

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Abstract: The European Chemical Agency (ECHA) encourages the use of *in silico* methods, provided that they are valid and this is demonstrated in an appropriate reporting format[1]. Several QSAR models covering various regulatory endpoints are freely/commercially available and have generated interest from the chemical industry. However, these models generally have been created for screening purposes and tend to lack the precision required to replace experimental studies. Recently, iSafeRat® toolbox has been developed to provide industry with high accuracy QSAR (HA-QSAR) predictions supported by appropriate documentation for their regulatory acceptance[2]. This work compares statistically the model performance of iSafeRat HA-QSARs with some other commonly used predictive models. To demonstrate the reliability of the iSafeRat modules, the authors will compare some high quality experimental data for several well-known industrial substances with predicted endpoints from the reviewed models. The comparative study will be supported by recognised statistical methods to clearly quantify the results. This work will also demonstrate how iSafeRat predictions satisfy the different OECD principles for model validation and accuracy making them suitable for use in REACH[3]. **Keywords:** model validation; QSAR; statistical comparison; REACH **References:** ECHA – Practical guide 5: How to report (Q)SARs. *Link:http://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf* iSafeRat® – *in Silico* Algorithms For Environmental Risk And Toxicity version 1.1 ECHA Guidance on information requirements and chemical safety assessment – Chapter R.6: QSARs and grouping of chemicals. *Link:http://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf*

Mechanistic effect modeling - beyond concentration response and constant environments (P)

WE233

Can survival of Gammarus pulex under four different exposure regimes of carbendazim be simulated with one toxicokinetic-toxicodynamic model parameterisation?

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The freshwater amphipod *Gammarus pulex* is known to be sensitive towards the fungicide carbendazim (Van Wijngaarden et al., 1998). A set of laboratory experiments was performed to investigate whether the sensitivity of *G. pulex* changed when facing different exposure regimes with different exposure durations of 2, 7, 14, and 21 days but identical time-weighted average (TWA) concentrations. For each exposure regime, 5 concentration levels were tested for effects on the survial of *G. pulex*. This data set offered the possibility to test the scope for a consistent parameterisation of toxicokinetic-toxicodynamic (TKTD) models to explain and predict mortality. We used the raw data on the survival of *G. pulex* over 21 days to estimate the parameters of two TKTD models, namely the reduced versions of the stochastic death (SD) and the individual tolerance (IT) model (Jager et al., 2011). We fitted the three model parameters of each of the two models for every single exposure regime separatedly and then for all exposure regimes at once. The parameter values estimated from the single exposure scenarios varied over several orders of magnitude. Parameter values estimated from all scenarios at once were always constrained by the corresponding parameter values from the single scenario estimations. Log-likelihood values resulting from the estimation using all data at once were for both the SD model and the IT model larger than the sum of the log-likelihood values from the single estimations, hence indicating a better fit resulting from all data at once. Our results show that the survial of *G. pulex* can be consistently simulated with one TKTD model parameterisation. The more data from different exposure regimes are used for parameterisation, the better are the TKTD fits and presumably is also the predictive power. Further, we compared predictions of mortality using an approach that combines the log-logistic dose-response relationship and TWA concentrations with the TKTD model approach. The TWA-based approach predicted high mortalities from the very beginning, in contrast to the TKTD model. TWA-based predictions of mortality are worst-case, but failed to match the time course of the survial of *G. pulex* as observed in the experiments. TKTD modelling performs better in prediction of survival over time.

WE234

Parameterisation of TKTD models to predict the survival of five aquatic species exposed to chlorpyrifos, pyrene and carbendazim

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Within the scope of the ChimERA project (http://www.cefic-lri.org/projects/38/21/), separate exposure and effect models will be combined into a chemical integrated exposure and effect ecosystem model for ecological risk assessment for the aquatic environment.
Toxicokinetic-toxicodynamic (TKTD) models are utilised to translate time-variable exposure concentrations into effects on the survival of aquatic invertebrates. For this study, we used historical and newly generated data sets on the survival over time for *Asellus aquaticus, Gammarus pulex, Brachionus sp., Chaoborus obscuripes,* and *Daphnia magna* from acute and chronic laboratory tests on the toxicity of chlorpyrifos, carbendazim, and pyrene to estimate the parameters of two TKTD models, namely the reduced versions of the stochastic death (SD) and the individual tolerance (IT) model (Jager et al., 2011). We implemented the parameter estimation in Mathematica 8.0 (Wolfram Research) and used the built-in optimisation routine *Simulated Annealing* to obtain parameter sets for the best fit between data and model simulations by maximizing the Log-Likelihood function. Not all combinations of compounds and species were tested and used for TKTD model calibration. Toxicity of the organophosphate insecticide chlorpyrifos to *C.obscuripes, G.pulex and D.magna* was evaluated*;* for the fungicide carbendazim all five species were tested, and for the polycyclic aromatic hydrocarbon (PAH) pyrene the toxicity to *D.magna, C.obscuripes* and *Brachionus sp*. was evaluated. The parameter values being estimated from survival tests will be useful to extend the knowledge about mechanistic links between exposure and effects to more species and compound classes such as e.g. PAHs. Within the ChimERA project, the parameterised models will be essential to couple time-variable exposure simulations to the survival in individual-based models

WE235

Damage-recovery modeling with Hill function for switch-like responses in tilapia exposed to pulsed waterborne copper

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The copper (Cu) pulsed frequency or dosing interval induced toxicity was not be fully investigated. Previous researches showed that damage dynamics with hazard behavior derived Hill coefficient could be used as an indicator to assess the adverse effects to aquatic organism in response to the constant metal exposure. The purpose of this study was to employ analytical and numerical methods incorporating with published data to investigate damage-recovery dynamics and biological switching to tilapia in response to pulsed waterborne Cu. We incorporated toxicokinetics/toxicodynamics with positive damage feedback model into system-level threshold damage model to examine the Cu susceptibility. Results show the threshold of Cu concentration at 50% average killing (k_kC50) and recovery (k_rC50) rates of whole body, gill, muscle, liver, and kidney increased with increasing dilution rate (k_0). On the contrary, threshold of dosing interval at 50% average killing ($k_k\tau$50) and recovery ($k_r\tau$50) rates increased with decreasing k_0. We found that ultrasensitivity appeared in whole body, gills, muscle, liver, and kidney with Hill coefficient $n \geq 7, 4, 7, 5,$ and 5, respectively, at Cu = 3 mg L^{-1}, $k_0 = 0.05$ h^{-1}, and $\tau = 72$ h, indicating that the positive damage feedback mechanism had been triggered. This finding reveals that the Hill coefficient *n* of gill will be a sensitive indicator rather than other tissues. We suggest that damage-time profile derived Hill coefficient of tissue could be used as a new risk indicator for species in response to fluctuating metals.

WE236

Predicting energetic consequences of toxic stress in holometabolic insects

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The responses of organisms to different types of toxic stress are metabolically costly, and have consequences for the individual's energy budget and life-history. These consequences, however, are difficult to predict not least of all because they lack a mechanistic framework for interpretation. Bioenergetics models provide a sound theoretical approach for estimating energy allocation in organisms by partitioning acquired energy among different processes. Using Dynamic Energy Budget Theory – a mechanistic theory that posits constraints on the metabolic organization of species - we formulate a full lifecycle bioenergetics model for holometabolic insects. The model captures the near exponential growth rate of insects, and the energetics of metamorphosis. We parameterize the model for the red flour beetle *Tribolium castaneum* (Herbst), a worldwide pest of stored products. We analyze toxic effects on the individual life-histories of *Tribolim castaneum* and interpret them as changes in model parameters induced by toxic exposure. Finally, we provide suggestions on how to make better use of bioenergetics models in ecological risk assessment. Our bioenergetics model is applicable to all holometabolic insects and provides useful theoretical predictions on the effects of toxicants in terms of energetic consequences.

WE237

Carbaryl toxicity prediction to soil organisms under high and low temperature regimes.

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The responses of organisms to chemical compounds may differ according to latitude and the predictability of the toxicity of chemical at different temperatures is important to risk management. Hence the toxicity effects of the pesticide carbaryl were evaluated at several temperature regimes, which are indicative of temperate and tropical countries, or characterizing predictions of temperature changes due to climate changes, or season temperature fluctuation. Four standard organisms (*Folsomia candida, Eisenia andrei; Triticum aestivum* and *Brassica rapa*) exposed to carbaryl under different temperature regimes and the effects assessed using synergistic ratios, calculated from EC/LC_{50} values and when possible the MIXTOX toll was used, based on the reference model independent action (IA) and possible deviations. The IA model showed to be a good tool to predict the toxicity of carbaryl at high and low temperatures for collembolan. Synergistic ratios showed a tendency to synergism at high temperatures for *Eisenia andrei* and *Brassica rapa* and antagonism at low temperatures in both species. *Triticum aestivum* showed less effect than expected (antagonism), when exposed to both low temperatures as high. This study highlights the need to include temperature regimes in risk assessment procedures depending on the latitude and also on sasonality. In addition, it is also shown that different species show different behaviours and patterns of toxicity regarding the influence of temperature on carbaryl toxicity.

WE238

Responses of Triticum aestivum and Brassica rapa to carbaryl and ultraviolet radiation.

M.R. Lima, Aveiro University / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology

The increase of ultraviolet radiation (UVR) reaching the earth's surface due to the increase of ozone layer depletion can affect crop yield, and in combination with pesticides used in agricultural activities can lead to greater risks to the environment. The impact of UVR and carbaryl singly and in combination on *Triticum aestivum* (wheat) and *Brassica rapa* (turnip) was studied. The combined exposure was analyzed using the MixTox tool and were based on the conceptual model of Independent Action (IA) and possible deviations to synergism or antagonism, dose-ratio or dose-level response pattern. Compared to the control, carbaryl and UVR individually led to reductions in growth, fresh and dry weight and water content for both species. The combined exposure of UVR and carbaryl induced higher deleterious effects than to the predicted by single exposure. For *T. aestivum* the pattern of response for plant length was based on additivity (no interactions between the two stressors; Independent Action); on the other hand, by using the weight parameters a dose-level deviation was observed at low dose levels of both stressors. The same pattern of dose-ratio was observed for length and dry weight of *B. rapa*, with UVR being the stressor responsible for synergism. An effect higher than expected (synergism) occurred when the endpoint analysed was the fresh weigh. Considering this, the present study highlights the importance of considering abiotic stressors like UVR in risk assessment.

WE239

INFLUENCE OF TEMPERATURE ON SUBLETHAL TOXICITY OF ZINC CHLORIDE TO SEA URCHIN EMBRYOS

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The toxic effects of chemicals can be influenced by abiotic factors including temperature in aquatic environment. In the light of increase use and production of toxic chemicals, additional stressors like global climate change might pose higher risk to aquatic organisms. We investigated the combined effects of temperature and zinc chloride on embryonic development of sea urchin, *Arbacia lixula*. Bioassays

were carried out at by utilizing sea urchin, *A. lixula* collected from natural seawater. Developing sea urchin embryos were exposed to zinc chloride (10^{-7} to $3x10^{-5}$ M) up to pluteus larval stage at 18 (standard temperature), 21 and 24 °C. Each experiment was run in six replicates. After each test, 100 embryo or larvae were scored as frequency of developmental defects on living individuals immobilised in 10^{-4}M chromium sulphate in each replicate. The following outcomes in embryogenesis were evaluated: a) normal pluteus larvae; b) retarded plutei; c) malformed plutei 1) exhibiting a number of skeletal or other abnormalities; d) developmentally arrested embryos; and e) dead plutei or embryos. Our study showed that toxicity of zinc chloride on *A. lixula* embryonic development increased with increasing temperature after at high zinc chloride concentrations (10^{-5}M $ZnCl_2$). Overall, zinc exposure resulted in a dramatic increase in abnormal embryos of sea uchins at high temperatures particularly in highly zinc polluted seawater compared with pristine environments.

WE240

Modelling the interaction between light intensity, nutrient concentration and oxidative stress in Lemna minor

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Metals are often found to induce the production of reactive oxygen species (ROS) in aquatic organisms. In plants, an overload of ROS can lead to oxidative stress, in turn leading to disturbances of the photosynthetic system. Since the light intensity determines the efficiency of the photosystems in plants, it can be expected to interact with oxidative stress. The nutrient concentration of the test medium determines the physiological state of the plant, affecting in turn the plant's capability of dealing with stress and hence influences the toxicity of the contaminant. We here present a mechanistic effect model for *Lemna minor* (common duckweed), which is based on Dynamic Energy Budget (DEB) theory. Models based on DEB have been used widely to study the effects of compounds on animals. Due to its general applicability to all types of organisms, it holds potential to be used for comparison of species and compounds in a broad context. Energy uptake from the environment is modeled explicitly, and metabolic rates are set to depend on temperature in DEB models. Therefore, they can be used to extrapolate effects to a wide range of environmentally relevant scenarios. Until now, the DEB research in ecotoxicology has focused on (heterotrophic) animals, where usually only one food source with constant composition is taken into account. Reproduction can in most cases be modeled simplistically as continuous production of offspring in the final developmental stage. A DEB model for a (photoautotrophic) plant should take into account both light and nutrients as energy input. Additionally, reproduction takes place differently than in animals (e.g., vegetative reproduction). Until now, no plant model based on DEB has been developed yet. We here present the first DEB model for a plant. It explicitly takes light as an input of energy into account, which enables us to study the interaction of light intensity and toxicity. As study organism, we chose *Lemna minor,* because of its advantages of being a relatively simple higher plant. We discuss the interaction of light intensity, nutrient concentration and oxidative stress using uranium toxicity as a case study.

WE241

Combined effects of copper and cyanobacteria on population dynamics of Daphnia magna under projected climate change conditions

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We conducted a 70 day exposure with a monoclonal *Daphnia magna* population (with an initial density of 5 neonates and 3 egg carrying females in 1L) with copper concentration (0, 45 and 90μg Cu/L) and a harmful algal bloom of *Microcystis aeruginosa* (absent or present) as the factors using 4 replicates per factor combination,. The expsore occured under imposed realistic seasonal trends of temperature and the amount of cyanobacteria (as % in total food). We explicitly contrasted current conditions with conditions projected to occur under climate change with two distinct temperature/cyanobacteria profiles (current vs. current+4°C temperature increase+50%increase in cyanobacteria in the the total diet). Every two weeks the temperature and the concentration of cyanobacteria (% of the total diet) were increased, while keeping the total food denisty constant. Under the current temperature/cyanobacteria profile the temperature increased from 15 to 19°C and cyanobacteria concentration increased from 10 to 20% in the total diet. Under the projected climate change temperature/cyanobacteria profile the temperature increased from 19 to 23°C and cyanobacteria concentration increased from 20 to 40% in the total diet. During the experiment, we monitored population dynamics, i.e. weekly records of population density (of three size classes) as a function of time. Comparison of the time series was used to make a direct assessment of how climate change (increasing temperature and cyanobacteria concentration of the diet) may alter the effect of copper on the population dynamics of *Daphnia magna*. Overall Cu without *Microcystis aeruginosa* had little effect on population density across all treatments. We noted complete extinction at the 90 μg/L and in the presence of *Microcystis aeruginosa* under the projected climate change scenario after 40 days while popualtions in the same treatment under the current climate scenario were on the verge of extinction after 70 days only. Under a combined exposure of 40μg Cu/L and *M. aeruginosa* we observed no signifcnat difference in population density with the control in the current climate scenario while the population declined signifcantly after 60 days in the projected climate change scenario. The long term goal of this population density time-series is to compare it against predictions from Individual Basel Models (IBMs) with the ultimate goal of integrating climate change aspects into ERA of chemicals.

WE242

Quantifying complex behaviour: The relevance of movement and its representation at different scales for estimating the recovery of aquatic macroinvertebrates

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In agro-ecosystems, organisms may be often exposed to anthropogenic stressors such as pesticides. The sensitivity and recovery of affected populations depend on toxicity, life-history, species' dispersal and landscape structure. Different testing strategies are applied for the ecological risk assessment of pesticides to understand potential environmental side effects of their application. Rarely, however, do standard tests account explicitly for the impact on animal movement and its relevance for recovery of populations from adverse effects. In the case aquatic macroinvertebrates (e.g. *Asellus aquaticus*), recovery by immigration of individuals from uncontaminated sites is an important factor for re-establishing population densities after pesticide exposure. Yet, not much information on movement patterns of aquatic macroinvertebrates is available nor which factors increase or decrease their dispersal potential. Hence, we performed video tracking experiments to derive information on movement behaviour of individual *A. aquaticus* under different conditions, such as varying population densities, pesticide exposure, and presence/absence of food and shelter. The obtained data set comprises information on resting times, straight move lengths, and preferred turning angle range under different experimental conditions, which we then used to parameterise a dispersal model. The complex correlation structures among and within the measured parameters caused deviances of the measured net displacements and those estimated according to the correlated random walk model. We tested different sampling protocols to identify the best representation for our test species. In combination with the MASTEP (Metapopulation model for Assessing Spatial and Temporal Effects of Pesticides) framework, it is possible to estimate recovery time frames for *A. aquaticus* assuming a higher degree of landscape heterogeneity than was possible in former studies.

WE243

Toxicant Mixtures: Ambiguities in the Determination of Toxicant Interactions

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Toxicants, whether natural constituents (*e.g.*, soil Al^{3+} or H^+), pollutants, or potentially intoxicating therapeutic drugs, often occur in mixtures. Toxicant mixtures may exert a total toxicity according to a toxicant-addition model (TA model) in which individual toxicant intensities (concentrations, activities, etc.) may be added or a response-multiplication model (RM model) in which the effects of individual toxicants may be multiplied. Within each model, toxicants may interact so that one toxicant enhances the toxicity of another (synergism) or reduces the toxicity of another (antagonism). The occurrence of toxicant interaction often cannot be demonstrated unambiguously because the appearance of interaction depends upon the model assumed (*i.e.*, TA or RM). Thus data from even well-designed experiments may conform well ($r^2 > 0.95$ in regression analyses) to two separate models: a TA model *without* toxicant interaction and to a RM model *with* toxicant interaction. In other experiments the data may conform well to a TA model *with* toxicant interaction and to a RM model *without* toxicant interaction. Thus arises the classic problem of differentiating between two apparently suitable but mutually exclusive hypotheses. Additional information regarding, for example, the mechanisms of action of each toxicant may help in selecting the more likely model. Nevertheless, ambiguities in the determination of toxicant interactions are likely to persist. We encourage experiments to subject their data to the regression models we present here in order to avoid making claims regarding toxicant interaction where the appearance of interaction, or non-interaction, is ambiguous.

WE244

Population dynamics of Paryale hawaensis associated to algae belt at Itanhaém beach, southeastern Brazil, as a tool for the establishment of possible "endpoints" for ecotoxicological analysis.

L. Alegretti, LEAL Laboratory of Ecotoxicology and Environmental Microbiology; M. Nicoletti Flynn, University of Campinas UNICAMP / LEAL Laboratory of Ecotoxicology and Environmental Microbiology

The size-class structure, density trends, sex-ratio, survivorship curve, net reproductive rate, generation time and per capita rate of population growth were established for a population of *Paryale hawaensis* from the intertidal region at the algae belt. Marine algae often forms habitat patches occupied by an associated fauna composed mainly by crustacean such as amphipods and isopods. Collections were taken once a month from Dec/2012 to Dec/2013. There was the presence of ovigerous females throughout the year imposing, with the continual reproduction, the overlapping of generations. A sex ratio biased in favor of females was recorded in all sampling dates, a common pattern in epifaunal species. *P. hawaensis* appears to be r strategist, with iteroparous females and multivoltine cycle. It has a Type II survivorship curve consistent with parental care. Preliminary reproductive potencial estimated from data sampled from December 2012 to March 2013 is 3,869, the generation time 4,89 and the per capita rate of population growth, 0,26. The production of small eggs is related to a decrease in maturation size of females, which allows the production of more than one brood per year. The reproductive strategy is related to the high-risk littoral habitat of the species which are exposed to variations in tide, osmotic pressure and thermal shock. Knowledge of the population dynamics of intertidal species constitutes an important tool to assess ecotoxicological damage in coastal areas. **Keywords:** Population dynamics, Ecotoxicology, *Parhyale hawaiensis*.

WE245

Accounting for adaptation in understanding population-level responses to anthropogenic stressors.

C.J. Salice, Texas Tech University / Environmental Toxicology

A primary objective in ecotoxicology is to understand and predict effects of chemical stressors on ecological receptors. Significant advances have been made with regard to this objective in that a series of mechanistic effects models have accurately predicted population dynamics of toxicant exposed laboratory populations. Many of the efforts, however, consider only single generation exposure and response or assume that trait responses to stress are consistent through time. Alternatively, research on evolutionary responses to environmental stress show that organisms are capable of rapid and significant adaptive responses that result in changes in mean trait values through time. For example, selection for more stressor tolerant individuals may change mean survival rates, which can significantly impact population dynamics. Hence, a significant challenge lies in exploring and incorporating adaptive responses into our understanding of stressor-impacted population dynamics. Here, I lay a foundation for using the concept of heritability to explore how adaptation can impact population responses to anthropogenic stressors. Narrow-sense heritability (hereafter, heritability) is the contribution of additive genetic variance to total phenotypic variance for a particular trait and is essentially a measure of adaptive potential. Realized heritability is the response to selection, R, divided by the strength of selection, S. In an ecotoxicological context, R is the difference in mean stressor tolerance between offspring and the entire parental generation and S is difference between the mean stressor tolerance of the selected parents compared to the entire parental generation. Differences in stressor tolerance can be expressed as changes in the LC50, time-to-death or percentage survival at a particular contaminant concentration. Heritability was incorporated into a stage-based population model of a freshwater gastropod as a change in survival rates through generations with heritability values ranging from 0.1-0.9. Preliminary results indicate the expected – as heritability increases, populations return to pre-stressor dynamics more quickly. If dispersal is included, however, this can dilute the effects of adaptive responses and slow the return of populations to pre-stressor conditions. This particular construct may be useful as an example for how to include adaptive responses in population-level assessments although considerably more research and effort is warranted.

WE246

An Individual Based Model of the Eurasian minnow – a virtual laboratory for extrapolating sub-lethal effects of pesticides to the population level

l. ibrahim; C.R. Hazlerigg, Imperial College / Division of Biology; T.G. Preuss, Bayer CropScience / Institute for Environmental Research; U. Hommen, Fraunhofer IME

This poster presents an individual-based model for the Eurasian minnow *Phoxinus phoxinus*. The purpose of this model is to reproduce the field population dynamics of the Eurasian minnow in edge-of-field streams in the European temperate region. The ultimate aim of building this model is to allow population-level risk assessment of pesticides on fish in edge-of-field water bodies especially when it comes to sub-lethal effects. The model species was carefully selected for this purpose to be a vulnerable representative of the field situation in terms of potential exposure to pesticides and population resilience to possible adverse effects of pesticides on reproduction. The modeled minnow population dynamics account for individual variability and sexual dimorphism in addition to interaction between individuals through size-dependent competition during mating and interaction between individuals and their environment through compensatory density dependent growth which in turn affects individual survival. Model design and parameterization are based on field data and demographic patterns which had been reported since 1940 and till this day, for minnow populations in general while focusing on temperate populations. Before the model can be used to extrapolate from results of ecotoxicological tests to field population it is first important to test the IBM on patterns observed for unaffected populations in the field so that pesticide case studies later can reflect potential effects with a certain degree of confidence. The poster presents the model description and validation results.

WE247

Coupled individual based models as a new tool to predict ecological effects in multi-species systems

K.P. Viaene, Ghent University / GhEnToxLab; A. Focks, Wageningen UR / Ecotoxicology Environmental Risk Assessment Team; h. baveco; P. van den Brink, AlterraWageningen UR / Aquatic Ecology and Water Quality Management Group b Alterra; F. De Laender, Université de Namur ASBL / Lab of EnvToxApplEcol; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology

The aim of ecological risk assessment of chemicals is to quantify the risk that a given chemical would adversely affect the structure and function of natural ecosystems. The ecological relevance of the currently used exposure and effect assessment approaches has increasingly been questioned in the last years. In response to this criticism, and given the large number of chemicals that need to be assessed, there is a need for cost-effective, ecologically more relevant alternatives to current approaches. Predictive modelling of chemical effects can possibly be used in this context. One of the challenges for the use of ecological models in this context is that virtually all modelling approaches developed in ecotoxicology focus on single species, whereas in reality ecological interactions between species will influence the way toxicants affect populations and communities. In this study, we propose coupled individual based models (IBMs) as a tool to assess chemical effects in communities. IBMs have the advantage of including variations in life-history characteristics at the individual level as well as being spatially explicit; hence they are ideally suited for ecotoxicological applications. By allowing single-species IBMs to interact with each other, ecotoxicological effects on single species under competition, indirect effects and effects on food webs can be assessed. The IBMs developed here have a generic structure and depend on a limited number of parameters only. Thus, IBMs can be easily applied and parameterised for a series of aquatic species. Effects of toxicants on individual-level endpoints such as mortality and reproduction are incorporated into IBM simulations using toxicokinetic-toxicodynamic (TKTD) model approaches. We present a case study where two IBMs for grazer species were coupled to a common resource (phytoplankton). The population dynamics of phytoplankton species were described using an ordinary differential equation. We evaluated whether the coupled species model was able to replicate patterns observed in experimental datasets, both alone, combined and combined under toxic pressure. We were also able to evaluate whether the coupling between exposure and effects needed to be described by detailed approaches such as TKTD, or if it was sufficient to use dose-response relationships.

WE248

Population modeling – Use of scenarios to avoid different levels of protection in current risk assessment

M. Wang, WSC Scientific GmbH / Dept Efate Modelling

In standard risk assessment of birds and mammals, simple calculations are used to estimate the risk posed by application of pesticides. Except for body weight and food intake, species-specific differences are not considered explicitly. In the present study, we show based on population modeling that this approach results in different levels of protection for different species or products. We show that landscape scenarios can be defined in a way, which results in a risk assessment with the same level of protection for different species, but such landscape scenarios need to be species specific. This is demonstrated using population models of small mammals, which are key species in current risk assessment.

WE249

An optimal Environmental Impact Assessment of Waste Water Treatment Plant: Risk Assessment & Predictive Tool

S. CASAS, Y. LIAO, VEOLIA Environnement Recherche et Innovation; A. MAITREJEAN, Veolia Eau

Coastal municipalities have to deal with numerous challenges concerning the supply and treatment of water, including large seasonal variations in demand, daily fluctuations in quality and volume and protection of environment. This project conducted by Veolia aims to develop a sound and complete methodology to quantify and monitor the potential environmental impacts that may occur in the marine environment related to the discharge of WWTP: to characterise the effluent and realize ecological habitat assessments. The selection and combination of environmental tools will be discussed for an optimal environmental impact assessment; capable of validating its environmental performance and efficient for the restoration of marine biodiversity. A part of the ecosystem monitoring program aims to provide a comprehensive and robust program of baseline and post

commissioning monitoring of marine environmental conditions to confirm quantitatively that there are no adverse effects to the marine environment resulting from the operation of the WWTP plant. It has been conducted in 1997, 2000, 2002 and 2006. 17 monitoring locations are categorized as follows: near outlet: positioned within the near field mixing zone, far outlet: positioned just at the edge of the mixing zone and near reference. Knowledge of the benthic littoral populations represents a fundamental tool for the conservation of biodiversity and its inclusion in the management of coastal areas (Rosenberg et al., 2004, Ruellet & Dauvin J.C., 2007). Information on the sensitivity and specificity of those environmental indicators provides a basis for planning the use and evaluating the potential environmental impacts. Once the biomonitoring data obtained, the project aims to understand the interactions within the littoral ecosystem to find the links between environmental factors monitored. A system of differential equations has been applied to describe predator - prey relationships in a food web and build an ecological model in continuous time. The numerical simulation results show generally three types of behaviour for the system dynamics: convergence towards a static state, a cyclic equilibrium or chaotic oscillations. Scenarios of change in nutrient flow are also available to managers to predict the future of the ecosystem in response to their control of the WWTP effluent.

WE250
Modelling impacts of environmental stressors and intra-/inter-specific interactions on shaping meiobenthos biodiversity in British estuaries
X. Sun, Institute of Integrative Biology; D.S. Lallias, University of Bangor / Molecular Ecology and Fisheries Genetics Laboratory; S. Creer, Bangor University; F. Falciani, The University of Birmingham / Institute of Integrative Biology
Investigating how multiple environmental stressors and intra-/inter- specific interactions shape the composition and spatial distribution of ecological communities is a challenging task in the risk assessment. Here we present a recent study in characterizing the meiobenthos biodiversity upon many physical and chemical stressors in Mersey River estuary and Thames River estuary of England. Both estuaries contain dozens of monitoring sites covering full salinity range from fresh water to saline water. Meiobenthic animals, normally used as bio-indicators on estuary water quality, are sampled from these sites and then characterised by DNA sequencing using a meta-barcoding approach. This analysis revealed a considerable ecological complexity representing 22 phyla and thousands of species. General environmental descriptors, such as temperature, wind speed, etc., and sediment water contaminants, are obtained from British Environment Agency, while the physical factors, e.g., shear bed stress and water flow velocity, etc., are obtained from hydrodynamics simulation. In total, over 20 environmental factors, along with organismic abundance data represented by 18,174 DNA sequence-based Operational Taxonomic Units (OTUs) in 35 monitoring sites, are included in our data analysis. The overarching objective of our approach was to develop and apply advanced computational methods to learn the structure of ecological networks representing the interaction between environmental stressors and species abundance. The combined use of statistical modelling and Bayesian networks have allowed achieving this goal and integrate existing ecological knowledge with the meta-barcoding dataset in a final representative model. Our research contributes a potential quantitative methodology to understanding environmental impacts on characterizing ecological communities.

WE251
Vitellogenin is not a suitable biomarker of crustacean feminisation
S. Short, G. Yang, University of Portsmouth; P. Kille, Cardiff University; A.T. Ford, University of Portsmouth / Biological Sciences
The expression of the yolk protein vitellogenin (Vtg) has long been used as a biomarker of feminisation in vertebrate species. Researchers have attempted to develop similar biomarkers to address whether reproductive endocrine disruption also occurs in the males of invertebrate groups such as the Crustacea. To date, the vast majority of studies investigating *Vtg* induction in male crustaceans have resulted in negative or inconsistent findings, leading researchers to question the utility of *Vtg* expression as a biomarker in this taxon. This study measured the expression of *Vtg* genes in two intersex phenotypes (termed internal and external) found in the male amphipod, *Echinogammarus marinus*, and compared them with those of normal males and females. Males presenting the external intersex phenotype are infected with known sex-distorting parasites and display clear evidence of feminisation, including oviduct structures on their testes and external female brood plates (oostegites). The internal intersex male phenotype displays a pronounced oviduct structure on the testes without the external intersex characteristics. This phenotype is not infected with known parasites and is possibly caused by environmental contamination. Given their morphology, these phenotypes are considered highly 'feminised' or 'de-masculinised' and can be utilised to test the suitability of biomarkers of feminisation. An *E. marinus* transcriptome was searched for genes resembling *Vtg* and two sequences were revealed, termed *Vtg1* and *Vtg2*. Results from a high-throughput transcriptomic sequencing screen of gonadal cDNA libraries suggested very low expression of *Vtg1* and *Vtg2* in normal males and both intersex male phenotypes. In contrast, the sequencing suggested notable levels of expression of both *Vtg* genes in females. Subsequent qPCR analysis validates these expression levels, with the signal for *Vtg1* and *Vtg2* transcripts in all male phenotypes being indistinguishable from that caused by contamination of trace levels of genomic DNA or the low-level amplification non-target sequences. Previously published results, in combination with the finding that *Vtg* expression is not notably induced in highly feminised amphipods, lead us to conclude that *Vtg* is not a suitable biomarker of feminisation/de-masculination in crustaceans and suggest the need for more appropriate biomarkers of feminisation in this taxon.

WE252
Influenza virus-host-environment interaction dynamics for assessment and control of respiratory disease risks
Y. Cheng, C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering
People spend most of their time indoors, which may increase the influenza exposure and infection risks. Moreover, influenza causes worldwide high morbidity and mortality. How to adequately quantify the indoor influenza transmission and implicate to associated respiratory disease risks control remains a primary issue. Therefore, the objective of this study was threefold: (i) to construct a virus-host-environment (V-H-E) interaction dynamic model for better understanding the underlying influenza transmission mechanisms indoors, (ii) to assess systematically the impact of respiratory disease risks influenced by V-H-E interactions, and (iii) to propose effective multi-control strategies to optimally mitigate influenza-associated respiratory disease risks. Results indicated that after experienced exposure time of 7.5 h, the mean pathogen number deposited in the respiratory tracts was 22158. By considering influenza virus replication and associated immune responses, the viral shedding distribution could be well described by a lognormal (LN) model with the geometric mean 5.02 logTCID50 mL^{-1} and a geometric standard deviation 1.12. We showed that it was likely (*i.e.*, 50% probability) that there were over 55% (95% CI: 40 – 70) populations being infected, indicating further control measures were needed to be implemented. It was likely that respiratory exacerbations risk would fall within moderate regime. Our study also showed that by two control measures of vaccine and surgical mask wearing could achieve complete influenza outbreak containment indoors. This study provides an integrated risk assessment framework to better assess the impact of respiratory disease risks influenced by V-H-E interactions and implicate to achieve optimal influenza outbreak containment in an indoor airspace.

Statistical challenges in ecotoxicology (P)

WE253
Challenging Responses from Ecotoxicity Experiments
J.W. Green, DuPont / Applied Statistics Group; T.A. Springer, Wildlife International Ltd
Ecotoxicity experiments include a wide variety of responses (endpoints) that require statistical analysis. A single experiment can have from1 to 25 or more responses and most include five or more. It is not possible to optimize experimental designs to be optimal for all responses. This leads to high variability for some responses, such as sexratio and VTG. Other responses have shallow concentration-response curves because of low levels of effects arising in part of water solubility limits of some chemicals. Moreover, some responses, such as histopathology severity scores, are inherently difficult to model. Physical limitations of laboratory equipment and space restrict the number of test concentrations that can be accommodated. All of these situations offer challenges to statistical modeling with the objective of obtaining meaningful estimates of effects concentrations (ECx). Examples of such situations arise in the Fish Sexual Development Test (FSDT), Fish Early Life Stage test (ELS), and Medaka Reproduction Test (MRT), among others. Computer simulations based on a large database of OECD validation studies and regulatory product submissions are presented to document the likelihood of modeling difficulties and alternative statistical evaluation strategies are offered.

WE254
Statistical Analysis of Aquatic Experiments Using Solvents
J.W. Green, DuPont / Applied Statistics Group
Aquatic toxicology experiments to determine the effects of chemicals sometimes require the use of a carrier solvent. Such experiments typically include both a negative (water) control group and a solvent control group. False positive rates and power to detect treatment effects in such experiments are compared for six possible strategies for deciding the appropriate control or controls for comparison. The evidence supports using either the solvent control only in all cases or a sequential strategy of combining the water and solvent controls unless the two controls are found to be statistically significantly different, in which case only the solvent control should be used. The elimination of the water control from acute experiments is considered to reduce the number of animals needed. This investigation rests on

computer simulations grounded in a large database of responses (endpoints) from a wide variety of experiments from numerous testing laboratories used in regulatory submissions and validation studies.

WE255
Breaking up is hard to do: Investigating the importance of breaking up algal chains in reducing the mean %CoV for section-by-section specific growth rate in Anabaena flos-aquae Toxicity Testing
A. Evans, Smithers Viscient ESG; K. Muddiman, Smithers Viscient
The OECD 201 Guideline details culturing and testing techniques recommended for performing environmental testing with freshwater algal species. However, meeting the validity criteria specified in this guideline can be difficult when testing with the cyanobacterium Anabaena flos-aquae, especially when compared to the more commonly tested algae species Pseudokirchneriella subcapitata. In particular, the mean coefficient of variation (%CoV) for section-by-section specific growth rate is often higher for A.flos-aquae than the 35% recommended in the guideline. Guidance is provided specifically for A.flos-aquae that includes testing at a lower light intensities (40-60 μE m-2s-1) than other algal species and placing test vessels on an orbital shaker table at 150 rpm. Specific guidance on inoculation density is also given. The document highlights the tendency for A.flos-aquae to develop "aggregates of nested chains of cells". Therefore, an important strategy for reducing variability in cell counts may be to break up these aggregates of nested chains. The objective of this investigation was to present data from a series of control replicates which, prior to counting, had undergone a series of techniques designed to break up cell aggregates and reduce overall chain length. Preliminary data indicated that reducing overall chain length may provide a more even distribution of A.flos-aquae cells across the counting chamber, thus reducing overall cell count variability and lowering the mean %CofV of section-by-section specific growth rate to acceptable levels.

WE256
Portion of time foraging (PT): How to avoid different levels of protection in the acute and chronic risk assessment
M. Wang, WSC Scientific GmbH / Dept Efate Modelling
In the current risk assessment of birds and mammals, a pragmatic approach is proposed by EFSA (2009) how to take the time an animal spends foraging (PT) in the crop into account: For the acute risk assessment, it is proposed to use the 90th percentile, in order to be "protective" in 90% of cases, and for the chronic (long-term) risk assessment, the mean is used, assuming an exposure period of 21 days, because it is unlikely that the habitat use of an animal will correspond to the worst-case on each day. While this approach was chosen in order to achieve a comparable level of protection for both the acute and the chronic risk, it is sometime proposed to use 90th percentile PT value also for the chronic risk assessment. Based on data from the field and using probabilistic simulations, we show which level of protection is in fact achieved with the different approaches. We show which PT percentile would be needed to achieve the same level of protection for both the acute and chronic risk assessment.

WE257
PROERA: A Multi-Criteria based Decision Support System for probabilistic ecological risk assessment
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The current environmental legislation (e.g. WFD, REACH) has increased the demand for ecological risk assessments of chemicals. Challenges emerge in the development of tools for the quantitative assessment of risk, where statistical and mathematical models have to be used, as multiple statistical aspects (e.g. ecotoxicological data analysis, estimation of reliability, uncertainty) have to be analysed. In this context, the European research project "AMORE" (Multi-Criteria Analysis for the Development of a Decision Support Tool for the prevention of Environmental Risks), funded by the National French Research Academy (ANR), aimed at the development of an innovative Decision Support System (DSS) able to perform probabilistic ecological risk assessment. The DSS is based on the use of Multi-Criteria Decision Analysis (MCDA). The objective of the DSS is to support the risk assessment approach in a reliable and reproducible way. It is a tool aiding in increasing the efficiency of risk assessment processes and allows the integration of different experts' opinions for supporting the process of structuring decisions. The DSS consists of the following 3 modules: Exposure Assessment – Predicted Environmental Concentration (PEC) graphs Effect Assessment – Species Sensitivity Distribution (SSD) graphs Risk Assessment - Potentially Affected Fraction (PAF) of species The innovative feature of the DSS is the incorporation of a new MCDA based Weight of Evidence (WoE) methodology, which aims to improve the evaluation of ecotoxicological data, through the assessment of their relevance and reliability for the definition of SSDs. The methodology is based on the assessment of a hierarchically structured set of 57 criteria, which is used for assigning a quantitative score to every ecotoxicological datum and was created based on the review of the state of the art frameworks for the assessment of ecotoxicological data. The different endpoints are analysed based on three main aspects: the 'Experimental Reliability', the 'Statistical Reliability' and the 'Biological Relevance' of the experimental protocol used during their production. The platform presentation reports the characteristics and the structure of the PROERA Decision Support System, the main mathematical details of the proposed WoE methodology for the analysis of ecotoxicological data, as well as the results of the application to selected case studies.

Policy assessment in an integrated systems perspective: indicators and targets to ensure operating within safe planetary boundaries (P)

WE258
Hybrid LCA approach for the assessment of greenhouse gas emissions of first and second ethanol production technologies in Brazil
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The understanding of greenhouse gas emissions of biofuel production is a key aspect to support the decision-making process concerning the environmental effects associated with the replacement of non-renewable fuels, given the current debate on climate change. In Brazil, several Life Cycle Assessment (LCA) studies have demonstrated the positive environmental effects of replacing fossil fuels – especially gasoline – with biofuels such as sugarcane ethanol (Macedo et al., 2008; Seabra et al., 2011; Cavalett et al., 2012). Most of these biofuel life-cycle inventories (LCI) have been compiled so far using a process-based approach which is in compliance with the International Organization for Standardization (ISO) 14000 series. However, the subjective determination of the system boundary can exclude some important processes which may result in underestimation in the LCI (Lave et al., 1995; Lenzen, 2002). In this sense, an input-output model can be used to describe a part of a product system and an ISO-compatible system boundary selection procedure can be designed by applying hybrid input-output assisted approaches (Suh et al., 2004). In this paper, the hybrid approach was carried out by inserting data on biorefinery scenarios from previous process-based LCA (Cavalett et al. 2012) into the input-output model as a group of new commodities and sectors. A tiered hybrid approach was performed to calculate the greenhouse gas emissions of first (1G-base and 1G-optimized) and integrated first and second generation (1G2G) ethanol production technologies in Brazil. Results reveal that 1G2G ethanol production is related to a lower global warming potential (measured in $CO_{2\text{-eq}}$/L) when compared with first generation ethanol production technologies. With the contribution of the input-output model, it was possible to identify both direct and indirect suppliers to the ethanol supply chain as well as their economic and environmental impacts throughout the Brazilian economy. Moreover, the results from hybrid method for greenhouse gas emissions is compared with those obtained by using process-based LCA approach, previously calculated by Cavalett et al. (2012). Such comparison is important to highlight the influence of the LCA approach on the results.

WE259
The potential role of LCA for the implementation and optimization of energy access projects?
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According to the International Energy Agency [1], about 1.3 billion people live without access to electricity and about 3 billion depend on traditional biomass as their main energy source. How to optimize implementation choices to provide an energy as clean as possible, without environmental burdens shifting, while ensuring production continuity, financial accessibility to local populations, and project viability? To go beyond the usual sole monitoring of green house gases emissions in the operational phase, a life cycle analysis (LCA) approach seems necessary to avoid the displacement of environmental impacts and provide multi-criteria analysis. LCA could also provide prospective quantitative evaluations to support virtuous projects in the fundraising phase. In this context, the environmental impacts due to several technologies used in electrification projects are modeled, for diesel generators (gensets), and for Solar Home Systems (SHS) with several batteries and PV panel options. A test case was built for an average level of off-grid consumption of 10 kWh per household per day in the rural Lira area (Uganda), which is equivalent to consuming on average 2 kW during 5 hours per day. The

model uses inventory data from the Ecoinvent v2.2 database (www.ecoinvent.org) and from the literature. The impacts were characterized with the IMPACT 2002+. The results obtained for off-grid electrification projects, highlight the need for an adequate End of Life battery treatment to ensure a better performance of off-grid SHS on most environmental indicators. To complete socio-economic approaches and technical constrains, an LCA based environmental evaluation parametrized tool could benefit energy access projects by providing key information for decision making at different project stages. It allows to highlight key areas of improvement and to compare alternative scenarios. Such tools should be handled with care and be integrated in broader regional strategic planning where global energy needs (from mobility to heat and electricity) are addressed in a systematic and sustainable way in order to take advantage from production systems complementarity.

WE260

Life-cycle impact of lignin on carbon, cost, and health from U.S. cellulosic biofuels

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Cellulosic biofuels have the potential to lower the carbon-intensity of automotive transportation, in large part due to the utilization of lignin for heat and power. While other components of herbaceous and woody biomass can be broken down into sugars and subsequently converted to a range of liquid fuels, the aromatic polymers that comprise lignin are more difficult to break down into high-value fuels or chemicals. Onsite combustion of lignin allows cellulosic biorefineries to largely avoid direct reliance on fossil fuels while also offsetting electricity generated by other, potentially fossil fuel-reliant power plants. The grid electricity offset credits alone reduce the life-cycle greenhouse gas (GHG) footprint of cellulosic ethanol by up to 20 g CO_2e/MJ ethanol, in some cases resulting in a net negative GHG footprint. To better understand these alternatives from a cost, energy, greenhouse gas (GHG), and human health perspective, we complete a life-cycle assessment (LCA) of four lignin utilization options: (1) onsite combustion for heat and power, (2) pelletization and export to coal-fired power plants, (3) co-firing and waste heat utilization at co-located coal-fired power plants, and (4) application as an SOC amendment. We first analyze the choices available to a single hypothetical biorefinery in the Midwestern United States, subsequently scaling the analysis up to a multi-biorefinery national scenario. Our national analysis is based on a U.S. cellulosic biofuel production scenario in which corn stover, wheat straw, and Miscanthus x giganteus are converted to 1.4 trillion MJ (60 billion liters) of ethanol annually by 2050, although the results are applicable to any biochemical process with similar energy needs that cannot break down lignin. We find that the results are highly sensitive to the chosen method for calculating co-product credits and the future sources of marginal power generation in the United States. We also find that, assuming efficient natural gas-fired power will supply most new electricity in the United States, co-locating biorefineries with existing coal-fired power plants (Scenario 3) has the potential to offer GHG benefits up to six times greater than Scenario 1, and 50% greater net GHG benefits relative to Scenario 2, but may have implications for the expected life of coal power plants otherwise slated to retire.

WE261

Between energy policy support and methodological research: a case study on the effective combination of equilibrium models with LCA to predict future impacts

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The potential effect of policy decisions is often tested against scenarios developed using equilibrium models, which are proven tools to forecast future socio-economic dynamics and impacts at the meso- to macro-scale of production process (e.g. partial equilibrium, PE) and regional system (e.g. computable general equilibrium, CGE), respectively. In some cases, PE and CGE have been used to predict environmental burdens associated e.g. with land use, water consumption or GHG emissions. However, the consistency of those projections is scarce due to the low representativeness and resolution of environmental extensions linked to the socio-economic system's model. Life Cycle Inventory datasets can be combined with PE or CGE to increase this granularity, but a few examples exist in the literature and no consensus has been yet reached. With this contribution, we report and discuss final results of a recent project that aimed at modelling future energy policy scenarios in Luxembourg, based on the combination between PE and CGE model's outputs with hybrid-Life Cycle Assessment (LCA). First, a PE model for energy sector was coupled with a computable general equilibrium model. The outputs of this combination were then used to build a hybrid LCA-based framework with prospective energy supply/demand data and economic input-output tables. This was implemented to assess potential environmental impacts (using the ReCiPe method) generated by Luxembourgish net consumptions over time. A "Business-As-Usual" scenario (BAU) was compared to a "GHG emission reduction" scenario (GHGr). Typical impact increases (~30% between 2010 and 2030) were observed in both BAU and GHGr due to the evident growth in net country consumptions, as concentrated particularly on climate change, and metal and fossil depletion. BAU impact scores resulted between 2,3% and 3% higher than GHGr scores. Concerning energy production technologies, most of impacts originated from the biggest plant in Luxembourg, which produces electricity from natural gas and whose remarkable contribution is constant over the two scenarios. Interestingly, the GHGr induced more imports of electricity, impacting other midpoints than climate change (e.g. ionising radiation). While perfect economic coupling did not occur between PE and CGE due to the embedded constraints and intrinsic modelling differences, the proposed approach is highly innovative and results are effective and suitable for policy support at national scale.

WE262

Production of electricity from forest residues in Portugal: environmental gains and losses using carbon footprint and LCA

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In Europe, the use of bioenergy is being promoted as a way to limit greenhouse gas emissions. In line with the European policy, in Portugal several power plants fuelled with forest residues have been built in the last few years. The number of power plants increased from two in 2006, with a total installed capacity of 12 MW, to ten power plants nowadays, with a total installed capacity of 106 MW. The construction of new power plants is planned in the short term. According to the National Strategy for Energy, the target is to achieve an installed capacity of 250 MW by 2020. However, is it correct to establish energy policies based on the reduction of a single impact? What happens to other impacts such as acidification or eutrophication? This study is focused in electricity production from two forest residues: cork oak pruning residues and eucalypt logging residues. Eucalypt and cork oak are the forest species that occupy the largest area in Portugal. The potential for reducing greenhouse gas emissions is evaluated by calculating the carbon footprint. In addition, a life cycle assessment (LCA) study is carried out in order to quantify other environmental impacts. A comparison with electricity production from natural gas and hard coal, the two most representative fossil fuels in the country, is performed. The system boundaries include the following stages: forest management, residue collection, chipping, loading and unloading operations, transportation of biomass, and energy conversion at the power plant. The functional unit is one kWh of electricity provided by the power plant to the national grid. Foreground inventory data are primary data representative of the current practices and technologies in Portugal. Background data are taken from Ecoinvent. The results show that electricity produced from forest residues has a lower carbon footprint than electricity produced from natural gas and coal. It is also favourable concerning abiotic depletion. However, for the remaining impact categories, there are environmental gains when the replaced fuel is hard coal, but there are environmental losses when the replaced fuel is natural gas. This study highlights the risk of establishing energy policies based only on the carbon footprint tool. A more comprehensive analysis should be also performed by using LCA as a complementary tool.

WE263

Integration of environmental criteria for public procurement of photovoltaic electricity

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Since 2008, the ESPACE-PV project, the Ecodesign of a photovoltaic system by conducting an analysis of its life cycle and its impact on the environment, was a project cofounded by ADEME and the partners involved (Cycleco, Transénergie, Mines Paris Tech). The main objective of this study has to bring scientific, technical and economical evidences on the positioning of photovoltaic in terms of environmental impacts. The results have contributed to emphasize the environmental impacts of each alternative and to identify, from this perspective, the improvement axis for industries involved and arguments to be taken into account by contracting authorities. In 2012, a methodological framework has been developped to perform environmental assessment of photovoltaic systems over their life cycle. It follows the principle that assessments of the environmental impacts of photovoltaics systems must take a life cycle and multi-criteria approach. In these instructions, default values – life cycle impact values and numeric value of process inputs - are proposed to the user for carrying out an Life Cycle Assessment study. This guide is available for industrial key players in early 2013 and will be used to assess the environmental impact of photovoltaics systems and could be used to support decision in French public policy related to photovoltaics. The method is based on a framework that defines the general scope of a Life Cycle Assessment study. The method for calculating the environmental impact of photovoltaics systems is detailed step by step. Finally this year, to implement this methodological framework, a web application has been developed. This software will be used by the key players of photovoltaics to perform its environmental assessment. The

French Government takes into account Life Cycle Assessment results in call for tenders as a significant criterion. \n

WE264

Environmental performance comparison between Pumped-Hydro Energy Storage and Battery Energy Storage System in Mexico

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Electricity generation from renewable energy has great potential in Mexico, however 69% of electricity comes from fossil fuels. Currently, the aim is to increase the electricity generation from wind power, geothermal, hydro and solar energy, which contribute to sustainability. To meet this challenge is required storage mechanisms that provide access to electricity when it is required and at the same time are technical, economical, environmental and socially viable. According to the last, in this study the environmental performance of two energy storage systems, considering the conditions of the Mexican Southeast, are compared: Battery Energy Storage Systems (BESS), represented by Lithium- ion Battery System versus Pumped -Hydro Energy Storage (PHES). Lithium- ion Battery System stores energy by the passing of ions between a lithium oxide (cathode) and a graphite anode, while PHES technology store energy based in the potential energy of the water stored in an upper reservoir, with the electricity generated by renewable energy (surplus electricity in off-peak demand hours), then the water is dropped, generating energy in peak demand hours. In this work the Life Cycle Assessment approach is used and the Life Cycle Inventory was generated considering the conceptual design of a PHES with a photovoltaic system developed for Tekax in Yucatan, Mexico. The Functional Unit refers to 3 MW of energy. The impact categories analyzed are: abiotic depletion, acidification, eutrophication, global warming, ozone layer depletion, human toxicity, fresh water aquatic ecotoxicity, marine aquatic ecotoxicity, terrestrial ecotoxicity and photochemical oxidation, according to CML 2000 method. The results indicate that the PHES generates lower environmental impacts for all categories, due mainly to the short useful life of the BESS (approximately 3000 charge cycles), while the PHES can be used during 100 years. Furthermore it is identified that the BESS efficiency decreases as the temperature increases, and because it is a tropical region must have air conditioning systems to permit an efficient operation. According to the results can be concluded that PHES associated with photovoltaic power generation is an environmentally viable system for the Southeastern of Mexico.

WE265

Assessing the sustainability of an innovative battery technology through the BiBAT project (LIFE+ 2012, Environment Policy and Governance)

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LIFE+ Environment Policy and Governance is one of the three Life+ European financial instruments for the environment put in place between 2007 and 2013. It supports innovative or demonstration projects within the spirit of the Göteborg strategy, in particular those contributing to the Lisbon process and Europe 2020, by adding measurable social and economic benefits to environmental benefits and building on a knowledge based approach. BiBAT project was accepted among LIFE + 2012 projects proposals and started in July 2013 for three years. It aims at developing a new Li-Ion battery generation with low environmental impact by the setting up of a prototype line. The battery developed within the project will address the "microhybrid" application, enabling the "stop and start" feature of recent cars. The emergence of the BiBAT technology can also stimulate the battery industry in Europe or worldwide for Electrical Energy storage linked to renewable energies (photovoltaics, wind power systems) growth. Up to now, the development of Li-ion batteries technology has been steered towards getting the best electrical performances possible. The environmental impact has always been considered as a secondary issue. In BiBAT project however, environmental evaluation and improvements of batteries for microhybrid application is the central action. It will drive the technical choices to be made in the project, will monitor the improvements in term of environmental impacts over the whole life of the product. The project will not only take an important step forward towards the environment, it will also seek an important socio-economic benefit. We will also follow a set of indicators in order to measure and potentially optimize this socio-economic impact. Since environmental, social and economic benefits and impacts are often interacting and interdependent, the sustainability of BiBAT's developments will be assessed as a whole instead of each aspect separately. \n The overall approach of the project will be presented. A review of existing method to assess sustainability will be conducted. Life cycle sustainability assessment and life cycle sustainability analysis (both abbreviated LCSA) as much as other assessment tools with a broad systems perspective will be considered. The most suitable to project objectives will be applied to assess together environmental, economic and social impacts and benefits of project developments.

WE266

Chemical storage for electricity surplus – a complementary possibility of physical storage?

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Introduction The increase of renewable sources for electricity production has induced several problems in terms of electricity management. The major problem is the intermittency of electricity production wich is not systematically consumed at the same time of its production. In order to use this produced electricity, it must be stored, usually using hydraulic storage.This system has the advantage of beeing well known and of giving back electricity when needed. Nevertheless, this physical storage needs a lot of space and the yield can vary a lot depending on the functioning of the pump. Another possibility is to store this excess of electricity using chemical processes. This electricity, obtained from renewable sources, can convert water into hydrogen and oxygen. Hydrogen can be converted in other chemical components which contain in another form the energy of electricity. That way of production for chemicals components can be considered as green, if biomass is used as source of carbon. **Materials and Methods** The aim of this study is to evaluate, in an environmental point of view, the potential of chemicals production (methanol, DME and SNG) using biomass and hydrogen obtained thanks to exceeeded electricity. Life Cycle Assessment methodology and ReCiPe method have been used. Two systems have been modelled for each product i.e. the gasification of biomass with H_2 produced thanks to exceeded electricity or without H_2. **Results** Gasification step, including the use of biomass assumed to be wood pellets, leads to the highest part of the impact, in each category for methanol production without external addition of H_2. This impact is mainly due to the preparation of wood pellets using electricity. When H_2 is used during the process, the amount of required biomass diminishes and allows a reduction of the percentage of gasification step for each impact category. The environmental impacts of the three studied products have been compared on an energy basis. The best score is obtained by SNG. **Conclusions** The impact of chemicals production is mainly due to the gasification step and more specifically to wood pellets production. Use of H_2 produced in a green process allows a high impact reduction in every category. It increases the yield and decreases the amount of biomass needed. Production of chemicals using exceeded energy could be another environmentally friendly way to store energy.

WE267

Hydrogen mobility life cycle assessment

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Hydrogen fuel cell vehicles are amongst the existing technologies for greener transportation, with its main advantage (compared to battery electric vehicle) residing in the autonomy, similar to current usage. ADEME, the Environment and Energy Management Agency, appointed Quantis to assess several hydrogen scenarios in the field of mobility through a comparative life cycle assessment (LCA) to contribute towards development of knowledge on this subject, mobilize industry players and contribute to the definition of the support policy for different hydrogen technologies. Two functional units are considered in this study: one kilometer-vehicle and one kilogram of output hydrogen from the producing site. Included in the scenario analysis is the hydrogen production from reforming of natural gas and biogas, but also from electrolysis while considering several electric mixes, one being the transition scenario "vision ADEME 2030-2050". Several transport distances and storage options are also assessed. Literature data have been adjusted and complemented with primary data that was provided by the industrial sector. The life cycle stages that contribute the most to the life cycle impact depend on the scenarios and indicators. For climate change, the impact is mainly from the production of H2 (for reforming scenarios) and from H2 transport to the distribution site (for scenarios with centralized production). For acidification, the production of the vehicle is dominant. The scenario with the overall best environmental performance is electrolysis on-site using a mix 100% wind. This is explained by the lower impact of the production (wind electricity) and the absence of hydrogen distribution by truck. Key improvement factors for H2 use in mobility include: reducing CH4 leakage for biogas production, reducing the electricity impact for electrolysis, lowering (or avoiding) H2 transportation distances, increasing H2 efficiency per km and enhancing the vehicle life span. The approach considered in this study does not allow taking into account the ability of different scenarios to meet the needs of mobility at a given scale. One possible follow-up to this study would be to evaluate different options for the deployment of hydrogen based on a scenario such as ADEME Vision 2030-2050 to systematically integrate the prospective dimension and matching the changing transportation needs and technology capacities.

WE269

Tiered decision framework for the development of sustainable chemical products and processes - Application to the biobased economy

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Designing benign chemicals is not an easy task, given production and use life cycle

aspects and a suite of pertinent regulations, environmental health and safety aspects, as well as transport and storage issues, next to economical and social aspects. Still, developments such as depletion of resources and climate change urge a transfer to a biobased economy. Biomass derived from living plants and algae and agricultural residues replaces fossil resources. The biomass is directly transformed into end products or biorefinery is used to create building blocks for the chemical industry. Biobased products and chemicals are not necessarily safe and sustainable by definition. To support decision making in the transfer to biobased substances and products, this contribution brings together a suite of relevant aspects. The aim of this study was to develop the key contours of a practicable tiered decision framework that integrates regulatory, environment health and safety (EHS) and sustainability criteria, for the whole life cycle of biobased substances and products. Grossly, the first tier of this framework is a quick scan assessment with a focus on the hazard characteristics of all chemicals produced, used or emitted during the life cycle. Veto indicators can be quickly recognized, such as carcinogenic, mutagenic and reprotoxic characteristics. Next. a second tier comprises a sustainability assessment using appropriately formatted summary indicators, such as a chemical footprint, the cumulative energy demand, and/or a water stress index. Apart from these, indicators which incorporate information about process economics and process and transport safety aspects are also included. When needed, the third tier includes more detailed information. Lower-tier steps can be skipped if detailed information is available. The comprehensive coverage of relevant issues, and the tiered format, simplifies and accelerates the discovery of key bottlenecks in designing benign processes and products, and identifies the most promising products and processes for further development. The decision framework enables innovators, risk assessors and policy makers to compare different (e.g. fossil based and biobased) substances and products along with production routes and gives insight into the possibilities of benign design and green chemistry.

WE270

Defining planetary boundaries for chemicals: a proposal for Persistent Organic Contaminants (POPs)

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The concept of the planetary boundaries to define a “Safe Operating Space for Humanity” was introduced by Rockström and coworkers in 2009. They identified nine environmental processes that may lead to unacceptable global changes: climate change, biodiversity loss, changes of nitrogen and phosphorus cycles, stratospheric ozone depletion, ocean acidification, global freshwater use, change in land use, atmospheric aerosol loading and chemical pollution. For most of these processes, quantitative parameters have been described and a quantitative boundary has been proposed in order to avoid irreversible global damage. This has not been done for chemical pollution. Defining a planetary boundary for chemical pollution (PBCP) is a difficult task considering the variability of emission patterns (entailing magnitude of emission and temporal and spatial aspects), environmental behavior and fate, type and intensity of effects of the numerous chemical substances released globally as a consequence of human activities. Developing methods for defining a PBCP are ongoing. A possibility could be defining boundaries for specific classes of chemical compounds. In particular, persistent organic pollutants (POPs) have similar features of environmental transport, are globally distributed, and are present in remote areas. Some of them are banned or controlled through international agreements, nevertheless, they are likely to persist for many decades. Moreover, new and emerging POPs, that are not yet controlled or regulated, are still used and their number is increasing. Knowledge concerning POPs,based on experimental data and theoretical models, is sufficient to allow for an estimate of global distribution and of exposure in remote areas. Particularly in remote cold areas, the risk for ecosystems as well as for human health may be high. A critical threshold for a planetary boundary may be set at a level of exposure above which unacceptable risk for adverse effects is likely to occur in remote areas (e.g. Arctic and Antarctic ecosystems). The approach may provide important insights regarding the effectiveness of preventive strategies to mitigate global chemical pollution (e.g. Stockholm Convention) as well as the need for future implementation of these control strategies for emerging chemicals.

WE271

LCA in support of more qualified air pollution abatement policies: Case of NMVOC emissions in European countries for 2000-2010

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Non-methane volatile organic compounds (NMVOC) cause human health damages through the toxicity exerted via direct inhalation of some substances – contributing to the impact category “human toxicity” in life cycle impact assessment (LCIA) – and through their indirect contribution to photochemical ozone formation (POF). To quantify these damages at national level and efficiently manage air pollution abatement initiatives, breakdowns of NMVOC emissions on individual contributing substances are required. In this study, we developed a methodology based on the combination of emission-source-specific substance distributions with total NMVOC emissions reported by European countries. Emission inventories including 270 substances could thus be built for 31 European countries for each year in the period 2000-2010, and then be translated into national impact scores for human toxicity and POF using commonly applied LCIA methods. It allowed us to (i) evaluate both the geographical differences within Europe and their evolution over time, and (ii) identify the major sources and substances with regard to impacts on human health, and (iii) appraise the relative magnitude of the direct and the indirect human health impacts associated with NMVOC emissions. Our results showed that POF impacts correlate well with the total NMVOC emissions, thus making the speciation of total NMVOC emissions unnecessary for these specific impacts and indicating that air pollution abatement policies aiming at reducing these specific impacts could rely on total NMVOC emissions. However, we demonstrate that the human health damage from toxicity of NMVOCs caused by direct inhalation largely dominate the human health damage from POF impacts and that they are largely caused by a very limited number of substances emitted from a few key sectors. Comparing NMVOC emission trends in European countries with the findings on human health damages associated with NMVOCs, we find that European air pollution abatement policies have not been efficient in this regard in the period 2000-2010 as some impacts on human health increased despite a decrease in the total NMVOC emissions. These findings support our recommendations to policy-makers to use tools or indicators that do not solely rely on emissions but also include impact assessment allowing for assisting a prioritization of the most contributing substances and sectors.

WE272

Determination of the environmental footprint of the anthropic water cycle using LCA

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This study describes the development of the life cycle assessment of drinking water in the Walloon Region, Belgium, starting from the pumping station up to the wastewater treatment plant. This study also includes rainwater harvesting as a complement to tap water. Data collected for this report are based on information available for 2010 as reference year. The functional unit is fixed at 1 m^3 of water used by a Walloon citizen (including both rainwater and drinking water). A model for the whole water cycle is elaborated in order to assess the impact of the different steps of the process, and to emphasize the importance of the different elements in each step. The model includes seven essential phases: collection and purification of surface and ground water, water main supply, rainwater harvest, sewers, municipal wastewater treatment plant, compact domestic wastewater treatment plants, and water elimination without any treatment. Two methods are used: ReCiPe 2008 and CML2002 (for eutrophication). Impact categories the more affected by the production and treatment of 1 m^3 of water are eutrophication, human toxicity, ecotoxicity, natural land transformation, and to a lesser extent ionizing radiation and mineral and fossil depletion. The more impacting steps are the collection and purification of water and the wastewater treatment in municipal plants. These steps are characterized by large consumption of electric energy and chemicals. Eutrophication is more directly linked to the elimination of wastewater without treatment. Mineral resource depletion is mostly due to the water mains supply. The less impacting steps are identified as rainwater harvesting, compact domestic wastewater treatment plants, and sewers. Impact of infrastructures is globally quite low because of the long term perspective of these plants, and the large amount of water that is concerned. Life cycle assessment of anthropic water pointed up the way that water consumption by Walloon citizens affects globally the environment. The most impacting steps of the process have been highlighted as well as the more affected impact categories. The survey of the way each step influences negatively ecosystems and sensitivity analysis have provided some approaches to improve the global environmental impact of human water consumption in the future.

WE273

LIFE CYCLE ASSESSMENT FOR SUSTAINABLE COCKLE FARM MANAGEMENT IN MALAYSIA

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Blood cockle, *Anadara granosa* sp, dominated about 80 % of shellfish production in Malaysia in the recent years. Currently, cockle farming covers approximately 10,000 hectares of mudflats area along the west coast of Peninsular Malaysia and involves about 1,000 cockle culturists among the local community.However,local cockle farm management is struggling with environmental threats to maintain its survival.In year 2011, the cockle production decreased dramatically about 50% compared to the previous year due to the high mortality rate of adult cockle.The fall off is viewed as a major threat to Malaysia as one of the major cockle producers in Southeast Asia.There is a crucial need to develop a comprehensive sustainable management plan for cockle farm.Sustainable cockle farming requires not only to identify the threats to cockle farming, but also to address the adverse effect of cockle farming towards the environment.Eventhough cockle farming has been portrayed as less harmful activity compared with other intensive aquaculture systems, a systematic environmental measures should be implemented to achieve sustainability goals hence to retain the safety and quality of this traditional species.A research framework has been set up as a preliminary step to promote LCA in local cockle farm management. Besides on-site field works to monitor current water quality parameters in the study area,relevant secondary data were collected to perform the proposed LCA research framework.The research framework comprises the main constituents in developing LCA framework for cockle farm management.The main activities in cockle farming have been identified and set to cradle-to-gate analysis.As cockle farming is considered as an open aquaculture system,system boundary has been made clearly as possible to distinguish the real environmental impacts derived from the cockle farming activity and the impact caused by the external threats.Besides developing the LCA model for cockle farming activity to support its sustainable production and management, this research initiates a way to raise the values and image of Malaysia’s cockle production in the global marketplace segment by enhancing its environmental performance.It is also create a basis to review the existing policies of Malaysia cockle farm management.From the other perspective, LCA can be regarded as an ideal environmental tool that may suit to all kinds of aquaculture systems and species across the regions.

WE274

Environmental Impact of Food Losses from Agriculture to Consumption in Switzerland

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Twenty to thirty percent of the environmental impact of consumption are caused by food consumption (Tukker et al., 2006). A key element to make our food system more efficient and sustainable is the reduction of food losses across the entire food value chain (Quested and Johnson, 2009). However, for the implementation of measures against food losses it is important to know which losses are environmentally most relevant. Beretta et al. (2013) quantifie the amount of food losses at the various levels of the Swiss food value chain (agricultural production, postharvest handling and trade, processing, food service industry, retail, and households) in terms of mass and energy. However, the environmental impact of food losses do not only depend on the amount of food loss, but also on the type of food, the degree of processing, the level in the food chain on which the losses occur, and on the method of treatment (incineration, composting, anaerobic digestion, feeding). This paper aims at quantifying the environmental impact of food losses at the various stages of the food value chain in Switzerland. Based on the mass and energy flow analysis, twenty-two food categories are modelled separately, representing the whole food basket. The analysis will be conducted with the software Simapro and EASETECH (http://www.easetech.dk/). Data on food production and processing will mainly be based on the Ecoinvent database and on available literature. For the impact assessment the categories *climate change*, *water*, and *land use* will be considered. The expected results are intended to help public and private decision makers to prioritize their strategies for preventing food losses and for optimizing the treatment methods of the remaining food losses. The assessment will distinguish food losses by food category, by stage of the food value chain, by method of treatment, and by reason. It will show hotspots of environmentally relevant food losses and evaluate the potential environmental benefits from prevention and optimization measures. Strategies to reduce food losses are as diverse as the reasons for the losses. This paper forms the basis to develop effective strategies to avoid food losses.

WE275

Developing eco-efficiency indicators for Swiss waste management

G. Meylan,

Swiss waste management is experiencing major technological developments aiming at better exploiting the energetic and material potential of waste. Sewage sludge will be diverted from cement production and municipal solid waste incineration (MSWI) to so-called mono-incinerators, so that ashes can be further processed to phosphorus fertilizer. In some MSWI plants, bottom ash already undergoes dry instead of wet discharge to enhance downstream metal recovery, while others are testing optimized acid washing of fly ash for the recovery of high purity zink. There are also initiatives and research carried out on further expanding source separation of plastic wastes. All these developments take place in different sectors of the economy which constitute the value chain of waste management from waste collection to preprocessing to final use of secondary raw materials. In this context, eco-efficiency indicators are of high interest for policy-makers. They provide insights into economic and ecological potentials and enable comparisons between waste management value chains. Moreover, progress within value chains or of Swiss waste management can be measured by eco-efficiency indicators. I propose an eco-efficiency indicator reflecting the environmental and economic benefits of waste management value chains over primary production by relying on environmentally-extented input-output analysis. The indicator is a vector defined for an input into an economic sector that that can come from primary or secondary production, i.e., waste management. Its first dimension denotes the environmental impacts avoided by using secondary raw materials per environmental impacts of primary production. The second dimension consists in the difference between economic value added of secondary production and that of primary production per value added of secondary production. Each dimension is adjusted for the actual recycling rate. The indicator on the input level can be aggregated into sector indicators (e.g., plastics production). Input indicator values are weighted according to the environmental and economic importance of primary input production prior to aggregation. The sector indicator shows how well waste management performs in terms of eco-efficiency for a specific sector. In turn, the sector indicator can be aggregated into an economy-wide indicator. Sector indicator values are weighted according to the environmental and economic importance of sectors prior to aggregation.

WE276

How can Collaborative LCA (Co-LCA) scheme empower a transition from Assessment to the Creation of Shared Benefits?

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Introducing innovative personal mobility will widely open up people opportunities to reach a more sustainable lifestyle. Carmakers shall implement LCA to support the imperative dialogue with their stakeholders. But actual developments focus on data inventory and impact assessment methodologies. Is it enough to assess a brand new technology system and carry fair comparison versus other products? Indeed no, nowadays, the biggest challenge for the practioner is the numerous decisions to take all along the study. The UNEP SETAC Life Cycle Initiative guidance propose a valuable progress path by involving stakeholders effectively in the decision making regarding key hypothesis, choices, interpretations. However, this is not any more a mathematical science question; this is community, collaboration, exchange and overall rewarding collaboration along the LCA performance. This work started in 2010 and now reaches its final level of rationalisation. New ways to conduct LCA are clarified according to the network relationships, this is the PEPS LCA maturity model: M1: PRESCRIBE – M2:EXPLAIN – M3:PARTICIPATE – M4: STIR UP. The research describe the management tools created to implement the Collaborative LCA (Co-LCA) which follows 5E steps : E1-EXPLORE the innovation peculiarity and detect lca issues, E2- ENGAGE a collective action with appropriate stakeholders; E3- ELUCIDATE the issue with specific tools & events; E4-EVALUATE the outcomes & benefits for participants and E5- EXTEND toward new lca routines & enrich partnerships. The electric vehicle LCA case study is tested to propose accurate management tools and limits based on experimentations involving in total more than 100 participants. Four main successes are the functional unit description, the selection of the right impact categories, the creation of inventories through meta-analysis and at last it gives a feedback and guidelines to build an original critical review panel of NGO and experts and redefine the role of each actor.\nIn final, the learning and obstacles are analysed and the benefits for each parties described to demonstrate the extra value to carry Collaborative LCA. Just like the first great navigator earned a lot of richness by gathering the specific resources of various overseas territories, it is bright clear that involving new multidisciplinary collaborator in the LCA routine does too.

WE279

Organizational Life Cycle Assessment – Guidance for organizations to conduct LCA considering their value chain

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In order to protect the environment in a credible manner, organizations need to rely on stable schemes. Several ones are available for organizations to assess the environmental performance of their products and services. Nevertheless, for the assessment at the organization level, the most applied and spread approaches have only recently considered the full value chain and mostly concentrate on a single aspect. While LCA was originally developed for products, the benefits and the potential of life cycle approach might be extended for organization assessment. However, an LCA for an organization appears to be even more complex. Recently, the “Organisation Environmental Footprint (OEF) Guide” was launched by the European comission and the ISO/NP TS 14072 for LCA of Organizations is under

development. Within this context, in April 2013, the UNEP/SETAC Life Cycle initiative launched the flagship project “LCA of Organizations”. The project aims to show that the application of the life cycle approach on organizations is relevant, meaningful and already possible. The nearly 60 participants of the project contribute to the drafting, consolidation and road-testing of a Guidance. The Guidance highlights the potential of organizational perspective within life cycle thinking and supports practitioners facing the main important methodological challenges when using Organization Life Cycle Assessment (O-LCA). O-LCA is a compilation and evaluation of the inputs, outputs and potential environmental impacts (considering a multi-impact approach) of the activities associated with the organization adopting a life cycle perspective. The three main items within the scope of O-LCA are reporting unit, reporting flow and organization system boundaries. Moreover, direct and indirect (both upstream and downstream) resources use and emissions should be taken into account in the study. The Guidance builds on existing internationally-agreed guides, methods and standards, particularly on the upcoming ISO/NP TS 14072. O-LCA is not intended to be applied for comparison of different organizations but for performance tracking. Because there is not a “one-size-fits-all” approach to O-LCA, several examples of organization’s pathways are presented with specific guidance for the application of O-LCA. The Guidance will be available for public consultation in few months. We would like to encourage you to provide feedback and help on moving it a step further.

Teaching and communicating sustainability – paving the way to a common understanding and meaningful actions (P)

WE281

Role and responsibilities of analysts in communicating Life Cycle Assessment results to decision makers: a case study in building sector

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Life Cycle Assessment (LCA) of building at early design phase is nowadays possible thanks to recent development in databases and tools. Nevertheless, we have noticed that the majority of building owners, architects and engineers don’t know how to deal with LCA results, how they can support decision and what the benefits of the LCA process are. The main questions they have are: ‘Indicators are too numerous, what are the most important ones?’ ‘What does the indicator mean?’ ‘How to deal with overlapping indicators (ex: ‘embodied energy’ and ‘resources depletion’)?’ and ‘What if some variant are better on one indicator and worse on the other?’. (Dammann, 2006) gives advices for an appropriate set of indicators for building owners: it must be short (no more than 3 indicators), complementary and exhaustive, easily understandable by building professionals and assessed thanks to a reliable LCIA method. If there were available, 3 reliable endpoint indicators on ‘Resources’, ‘Ecosystem Quality’ and ‘Human health’ would probably be the best for decision support. In addition, several other parameters have to be taken into account in the decision process as, for instance, comfort and living condition, aesthetic and contribution to local socio-economic vitality. A solution to integrate most of sustainability aspects could be to complete a Life Cycle Sustainability Assessment (LSCA) but research on that topic in still on-going. Waiting for research progress on Endpoint assessment and LCSA, analysts still need to communicate LCA results to decisions makers avoiding loss of credibility and mistrust in LCA method and benefits. Cycleco proposes a step by step method for LCA results analysis and interpretation once Life Cycle Impact Assessment using ILCD recommended models has been performed. The method recommends drawing first conclusions on Climate change impact which is considered as the most important indicator. Then a procedure for refining result and conducting sensitivity analysis is done, in particular using the 14 other impact indicators and taking into account the fact that stakeholders always have an *a priori* favourite scenario. At the end a one page results report is built for supporting building owners and designers’ decision. The step by step method is implemented on a comparative LCA of biosourced constructive systems as an illustration.

WE282

Defining, Quantifying and Teaching “Sustainable Nanotechnology”

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Sustainable nanotechnology is being touted as a concept that can guide incremental nanotechnology development amidst significant data gaps and uncertainty. The best-known definition of sustainability has been formulated by the Brundtland Commission,andpoints at the broad societal needs of social equity, environmental protection, and economic stability. The Triple Bottom Line (TBL) definitions of sustainability envision the environment, society, and economy as three pillars of sustainability. While the BrundtlandCommission’s definition espouses a universally cherished vision, it has intrinsic limitations in addressing specific problems such as those related to the production and use of nanomaterials. On the other hand, TBL requires consideration of each pillar and the interactions between them, and can, in principle, systematically “trickle down” to technology design details to ensure a sustainable nanotechnology enterprise. We report the results of a pedagogical intervention to assess the application of sustainability criteria to nanotechnology selection using the TBL approach. A project course was developed at Ca’ Foscari University of Venice (Italy) in the Fall of 2013. Twenty students were tasked with conducting a literature search on Sustainable Nanotechnology definitions and operationalizing the TBL approach for Sustainable Nanotechnology. Though the volume of literature containing an association between sustainability and nanotechnology was significant, the search yielded no concise definitions for sustainable nanotechnology.The class then developed a Multi Criteria Decision Analysis (MCDA) modelthat allowed explicit integration of technology performance on selected criteria and stakeholder values.The students developed a sensitivity analysis which demonstrated how stakeholders made trade-offs by switching from high-end to low-end alternatives as they weighted criteria beyond a threshold. The literature review and model results call for a detailed and pragmatic articulation of the concept of Sustainable Nanotechnology to better bridge the divergence in knowledge and stakeholder values. Our pilot pedagogical experiment has made an inroad into this effort, though much work remains to develop this decision tree further. **Keywords:** Sustainable Nanotechnology, Multi Criteria Decision Analysis, Case-based Teaching

WE283

Implementing a Life Cycle Management capability maturity model at an Indian chemicals company

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As part of the capacity building efforts supported by United Nations Environment Programme in developing countries to mainstream Life Cycle Approaches, Federation of Indian Chambers of Commerce and Industry is working with an Indian chemicals company on a pilot project to implement the Life Cycle Management Capability Maturity Model (LCM-CMM). The company has already done Life Cycle Assessment on one of its products and is now working to undertake Life Cycle Assessment for additional products as well as to enhance the quality of the assessments and benchmark them against global standards. This is the prime driver leading them to take up a pilot project with technical support from United Nations Environment Programme. The LCM-CMM pilot project was initiated by conducting a training workshop for which the standard CMM matrix and questions were thoroughly studied and a customized questionnaire was developed to suit their specific context. The training workshop conducted over two days covered two aspects: (i) Explanation of LCM-CMM requirements at various maturity levels (training by consultants) and (ii) Connecting these requirements with how Life Cycle Management is being handled at the plant (training by internal resources. The participants were asked to fill in a questionnaire that could eventually become the basis of current maturity level assessment. The questionnaire was filled in by the participants before and after part (ii) of the training. The participants were also asked to give comments on their choice of answers which were used to further analyze how well the participants have understood the application of LCM-CMM model. This paper will show how LCM-CMM is being implemented in a chemical company based in a developing country. Starting with a situation where life cycle assessment work was primarily undertaken to satisfy demands of international customers, the company now understands how this work can be done in a way that benefits the company. The whole issue was approached with a strategic intent with a supporting framework that provides a structured and systematic way of developing skills and capabilities systematically at individual, team, organization, and value chain levels.

Persistent, Bioaccumulative and Toxic (PBT) substances – identification, assessment and regulatory decision making with a special focus on socio-economic aspects (P)

WE284

Incorporating Environmental Impacts in the Benefit –Risk Evaluation of Veterinary Medicinal Products (VMP)

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For Veterinary Medicinal Products (VMP) the outcome of the Environmental Risk Assessment (ERA) during the authorization process is part of the benefit-risk evaluation and the pharmacovigilance system. The ERA may result in the conclusion that no risk is expected or that the product poses a risk for the environment (risk quotient > 1). It may further be concluded that it enters groundwater at relevant concentrations (> 0.1 μg/l) and/or that the active ingredient is a persistent, bioaccumulative and toxic (PBT) substance. To date in benefit-risk evaluations of VMPs, these risks for the environment are only considered individually and qualitatively. We will present first results of an ongoing project about the possibilities to conduct a benefit-risk evaluation in case of serious environmental concerns for VMPs by considering socio-economic analysis (SEA) and ecological impacts. It is the aim of the project to develop a general concept on how potential environmental impacts can be adequately described in the benefit-risk evaluation. The following approaches from other fields of authorization are assessed with the intention of transferring suitable procedures to VMPs: Benefit-risk evaluation for veterinary pharmaceuticals in other relevant fields like undesirable effects versus clinical benefits (EMA) Benefit-risk evaluation for human pharmaceuticals (EMA) Authorization of chemicals in REACH (ECHA) Authorization of plant protection products (EFSA) Authorization of Biocides (JRC/ECHA) The developed concept will be tested for selected VMPs, where environmental problems are known and a reliable database is available. Furthermore we will work out criteria for substituting active ingredients. Which criteria can be used for classifying substance as potential substitution candidates because of environmental concerns? In which cases is a substitution ecologically worthwhile? Further possibilities on how to handle substances with substantial ecological risks but high benefits will be discussed. Possible risk management plans for VMPs will be developed, analyzing the role of eco-pharmacovigilance and specific risk mitigation measures.

WE285

Sensitivity comparison of fish and daphnia toxicity - evaluation of ecotoxicological testing strategies and chronic fish testing

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For environmental risk assessment predicted no effect concentrations for each compartment are derived representing concentrations below which no unacceptable effects are expected. The PNECs are usually derived from laboratory effect studies by applying an assessment factor on the lowest determined effect value. With regard to the aquatic compartment experimental data on chronic fish toxicity are still limited, and an increased range of acute to chronic ratio is expected as fish studies cover different species, exposure times and endpoints. Moreover, the question arises whether the chronic toxicity of fish can be adequately covered by other trophic levels as species specific mechanisms of toxicity such as effects on the endocrine system or on developmental stages may contribute to the toxicity of a chemical. At the same time animal testing should be avoided. In conclusion, are the existing testing strategies adequate, and in which case is chronic fish testing necessary? The aim of this study is an evaluation of the integrated test strategy according to REACH guidance and a review of the reliability of conclusions from acute to chronic effects of chemicals on the basis of a data analysis of already existing toxicity tests on Daphnia and fish. Acute and chronic studies from about 250 experimental datasets for both trophic levels were evaluated. The dataset is based on data from the OECD eChemPortal and from the Information System Chemical Safety database (ICS) of the German Federal Environment Agency. Only studies conducted according to the European Union Technical Guidance Documents were considered. ACR correlation across both trophic levels as well as a correlation between the toxicity level and baseline toxicity based on the octanol-water partition coefficient is investigated. Based on species sensitivity comparison of acute tests the results of the data analysis indicate that chronic fish testing can be avoided in most cases without underestimated environmentally hazardous substances. But chronic fish testing will still be required for a protective risk assessment. The presented classification scheme may contribute to a scientifically justified testing strategy approach.

WE286

AIST-MeRAM: an easy-to-use tool for aquatic environmental risk assessment and management of chemicals

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A momentum toward risk assessment and management of all chemical substances is gathering strength with the enforcement of REACH in EU countries and the revision of KASHINHOU in Japan. Due to the complicated procedures and the required advanced methodologies-and-data in ecological risk assessment, the management of chemicals from the standpoint of ecosystem impact still is very few. The aim of our study is to provide national regulatory agencies, industry and academia with state-of-the-art and easy-to-use ecological risk assessment tools for better research, analysis and visualisation purposes for use in risk assessment and management of chemicals, and in preparation of regulatory filings such as Kashinhou, REACH etc. All of the methodologies of ecological risk assessment including Hazard Quotient, Species Sensitivity Distribution, and population-level impact assessments, together with all of the ecotoxicity test data are built-in with an easy-to-use interface. The demonstration of the tool will be introduced on the meeting.

WE287

How to avoid dangerous alternative to banned chemicals in a benign by design approach: screening of Flame Retardants by the cumulative PBT-Index in QSARINS

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During the last decades, Brominated Flame Retardants (BFRs) are widely used in various industrial products in order to prevent or slow down a fire. The use of some of these BFRs, such as polybrominated diphenyl ethers (PBDEs), is now restricted or prohibited under various legislations, due to their hazardous properties regarding Persistence, Bioaccumulation and Toxicity (PBT properties). PBDEs are nowadays replaced by other different New Flame Retardants (NFRs), such as new BFRs (NBFRs) or OPFRs (OrganoPhosphorous Flame Retardants). Nevertheless, the information about the chemical properties of these substitutes are often not available and these substances were commercialized without complete information regarding the PBT properties, that are based on long-term behaviors and require complex, expensive, prolonged experiments. However, the PBT assessment is expressly required in the context of REACH regulation and PBT chemicals require an authorization. In this study, several new compounds, proposed and used as ”safer alternatives” to PBDEs, such as NBFRs and OPFRs, were analyzed with multivariate evaluation tools and screened with the cumulative PBT-Index model, implemented in QSARINS (QSAR-INSUBRIA), a new software for the development and validation of multiple linear regression (MLR-OLS) Quantitative Structure-Activity Relationship (QSAR) models. A rigorous check of the chemicals that are included in the model Applicability Domain, and for this reason with the most reliable predictions, has been done. The results, obtained directly from the chemical structure for the three properties altogether, have been compared with those obtained by the US-EPA PBT profiler and a good agreement among the two different approaches was found, supporting the utility of a consensus approach in these screenings. A priority list of the most dangerous chemicals has been proposed, highlighting that some substitute-compounds are detected as intrinsically hazardous for their PBT properties. Moreover, this study also shows that the PBT-Index model could be a valid decision-making tool in the socio-economic assessment (SEA) to evaluate appropriate and safer substitutes, immediately from the chemical design, in a benign by design approach, thus avoiding unnecessary and expensive synthesis and tests.

WE288

Method development to screen and identify new persistent, bioaccumulative, toxic (PBT) substances

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Research and development provides us with an abundance of substances that can be used in a wide array of applications. Some of these substances can be harmful to the environment. An evaluation method is presented to identify these environmentally harmful substances. Emphasis is being placed on potential persistent, bioaccumulative, toxic (PBT) or very persistent, very bioaccumulative (vPvB) substances. The identified substances are categorized according to their PBT potential and a methodology is presented to screen them in an effective way for further evaluation. This paper takes into account already existing screening methods and PBT treshold values by various regulatory bodies as well as industries. A wide range of substances with available endpoint data are sorted according to a calculation and prioritization model. Full evaluation of a substance may take months. In order to concentrate these efforts on the most promising substances a pre-screening model is presented which uses Open Source estimation programs, quantitative structure-activity relationship models (QSARs) as well as literature and online based research to identify their PBT potential with a certain accuracy within a matter of hours. Challenges in the process of PBT-identification are highlighted.

WE289

Steps needed to incorporate scientific developments into regulatory practice – needs to improve the PBT/vPvB assessment

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Chemicals Agency (ECHA)
The amendment to Annex XIII (Criteria for the identification of PBT and vPvB substances) to REACH by Commission Regulation (EU) No 253/2011 entered into force on 19 March 2011. In addition to the information directly numerically comparable with the legal criteria (degradation half-lives from simulation tests, BCFs from aquatic bioaccumulation tests, aquatic chronic test results) the use of other types of information in the PBT/vPvB assessment has been made formally possible by the amendment. Some science based approaches for using such other information have been developed in the past and are already reflected in ECHA Guidance. However, there are several areas where the understanding on how other information can be used in the PBT/vPvB assessment still needs to be developed in research and/or in the regulatory context. The incorporation of scientific developments into regulatory practice requires several work steps, including scientific review of existing approaches and gap analysis, in-depth analysis of the regulatory context, review of precedent cases, benchmarking, discussion with relevant stakeholders, and guidance development for regulatory purposes. ECHA and ECHA's PBT Expert Group have identified several issues for which such method development work is needed. These cover all endpoints P, B, and T (environmental effects and human health effects). Selected issues and the work steps envisaged to tackle them in view of application in the regulatory context will be presented more in detail.

WE290
Main findings of the ECETOC Task Force on PBT Criteria (Annex XIII of REACH)
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In 2013 an ECETOC Task Force on REACH ANNEX XIII PBT criteria was formed. This Task Force has reviewed and analysed the scientific literature to determine the environmental and human health relevance of the amended REACH Annex XIII assessment information. Furthermore, the group has identified the advantages, disadvantages and difficulties associated with the application of the new assessment information and to develop guidance as to what screening information is sufficient for a decision to determine whether a substance fullfils the criteria, based on existing guidance. The Task Force aims to develop integrated evaluation strategies for the most common outcomes of the screening assessments where a PBT/vPvB conclusion cannot be reached, based on the available data. Specifically, the Task Force has addressed factors influencing the results of field studies on biomagnification and trophic magnification factors (BMFs, TMFs), as well as laboratory studies on BMFs. Furthermore, advice is given on how to interpret biomonitoring (BM) information with regard to the criteria. Finally, the need for further research has been identified in order to develop alternative information to assess persistence, bioaccumulation and toxicity. The poster will present the main findings of this Task Force.

WE291
Effectiveness of measures adopted for the reduction of nonylphenol concentrations in European rivers: the case study of river Lambro, Northern Italy
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The present work analyzed monitoring data on nonylphenol and their short chain precursors (nonylphenol mono- and di-ethoxylates, NPE1 and NPE2) collected in a highly impacted river basin in Northern Italy in order to assess the effectiveness of the adopted measures, during two monitoring campaigns in 2003/04 and 2009/10, respectively before and after the entry into operation of three new WWTPs of the town of Milan. The study area was the basin of the river Lambro which flows through the most urbanised and industrialised area of Italy, collecting discharge water also from the town of Milan. River Lambro is the main source of pollutants also for river Po and the Adriatic sea. The availability of data collected in stations subject to different pressures and where different measures have been undertaken allowed us to differentiate the effectiveness of the various reduction approaches. Data collected in the present work showed that in the last seven years the reduction of the different analytes was from about 70% for NP to 90% for NPE2. Comparison between the annual averages of 2003/04 and 2009/10 shows a substantial reduction in the concentration also upstream Milan. In the first campaign the mean values measured up- and downstream Milan were 1.35 μg/L and 1.20 μg/L, while in the second campaign the mean values were 0.34 μg/L and 0.15 μg/L respectively. At the first station the decrease can be attributed to the reduction in the use of the substances, while in the second one should be added the NP decrease attributable to the entry into operation of the new WWTPs of Milan. By comparing data in the various monitoring stations in the basin we can conclude that the most of the reduction can be ascribable to the substitution of NPE in the industrial uses as demonstrated by a survey in the mostly industrialized area of the basin, characterized by a textile enterprise cluster, which discharges in the tributary Olona. The entry into operation of new WWTPs for the treatment of urban sewage of Milan contributes for about 10% to the reduction of the total NP load discharged by the river Lambro. The plant efficiency in sedimentation reduces the supply of SPM to the river from the town and, on the consequence, fraction of NP bound to particles. Thereby nonylphenol compounds discharged by river Lambro are for more than 90% in the dissolved phase, reducing also the risk of nonylphenol deposition in the river Po sediment during the mixing of the two river waters.

WE292
Fluoroalkyl Substances and Flame Retardants in Air in the Canadian Arctic and Great Lakes Basin
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Two major air monitoring programs conduct measurements of persistent organic pollutants (POPs) and other priority organic pollutants in the Canadian Arctic and the Great Lakes regions since the 1990s. At the Canadian High Arctic station of Alert, Ellesmere Island (82°30' N, 62°20' W), POPs measurements in air started in 1992 under the Northern Contaminants Program (NCP). Air monitoring of POPs has also been conducted in the Canadian Great Lakes Basin (GLB) since 1990. Measurement data from the GLB contribute to the Integrated Atmospheric Deposition Network (IADN), a collaboration between Canada and the United States. There were two Canadian GLB master stations, located at Point Petre (PPT) on Lake Ontario (43°50' N, 77°09'W) and Burnt Island (BNT) on Lake Huron (45°49' N, 82°57' W). The two monitoring programs have been measuring the air concentrations of polybrominated biphenyl ethers (PBDEs) in Arctic and Great Lakes air since 2002 and 2005, respectively. Air samples collected from both the Arctic and the Great Lakes have also been screened for non-BDE flame retardants (FRs) since 2007 and 2008, respectively. Alert air samples were screened for per- and polyfluoroalkyl substances (PFASs) since August 2006. Time trends of PBDEs, non-BDE FRs and PFCs were derived from these measurements. It was found that PBDEs were declining in the GLB but not at Alert until after 2006 implying transport from regions other than North America to the Arctic site. Several new FRs were detectable in both GLB and Arctic air. Perfluorooctane sulphonate (PFOS) precursors, methyl and ethyl perfluorooctane sulfonamide (MeFOSE and EtFOSE), showed non-changing and declining tendencies, respectively, at Alert from 2006 to 2012. In contrast, perfluoro alkyl carboxylate (PFCA) precursors, 6:2, 8:2 and 10:2 fluorotelomer alcohols (FTOHs), which were not regulated at the time of measurement, showed increasing tendencies in air at Alert. The presence of new and emerging organic pollutants in air at remote locations in the Canadian Arctic and Great Lakes regions imply long-range atmospheric transport potential of these compounds; indicating the need to further investigate their environmental fate and toxicity.

WE293
Articles with POP-BDEs: recycling or incineration?
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In 2009 commercial penta- and octa bromodiphenyl ether (BDE) were added to the Stockholm Convention on POPs with the ultimate aim to phase out these substances. Production and use is now forbidden, but an exemption was introduced for recycling articles containing the listed POP-BDEs: tetra- and pentaBDEs present in commercial pentaBDE, and hexa- and heptaBDEs present in commercial octaBDE. Recycling appeals to the transition towards a circular economy. Parties using the exemption are, however, are obliged to evaluate its need regularly. A study was carried out to investigate the BDE-flows in various automotive and the electric/electronic waste streams in the Netherlands. The reason is that commercial penta- and octaBDE were most applied in these sectors according to the literature. The total amounts of POP-BDEs circulating within these waste streams were estimated. The measurements showed that the electric/electronic polymer waste flow was much larger than that of the automotive sector, but also that it contained considerably more POP-BDEs: average POP-BDE concentrations, based on mass balance, were ten times higher. These concentrations were well below the POP-BDE concentration limits proposed by the European Commission in 2013.

However, individual articles in the waste streams could exhibit concentrations exceeding the proposed limits. The findings pose a challenge to policy makers who have to decide between recycling the polymers, including the POP-BDEs, or environmental sound disposal of the polymers . There may also be an intermediate solution in which POP-BDE-rich articles are sorted out and disposed of, whereas the remaining polymers are recycled. At present, such an intermediate solution is hindered because a rapid, on-site analytical method for detecting the different congeners in the waste streams is not yet available. Furthermore, an overview of articles which may contain the POP-BDEs in relevant concentrations is also lacking. Knowledge of the recycling sector and feasibility of the various options are important keys in finding the most favorable solution.

WE294
Baseline of Chemical assessment of PCBs, POPs and PAHs in sediment and sea anemones on natural populations from different climatic scenariou
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In the European Union (EU) about 3000 different substances are used in human medicine such as analgesics and antiinflammatory drugs, contraceptives, antibiotics, beta-blockers, lipid regulators, neuroactive compounds and many others. Also a large number of pharmaceuticals are used in veterinary medicine, among them antibiotics and anti-inflammatory chemicals. In the last few years, knowledge about the marine and coastal environmental occurrence of pharmaceuticals and other pollutants has increased to a large extent due to new analytical techniques able to determine polar compounds at trace quantities. The assessment of bioaccumulations values in complex environmental mixtures requires application of integrative procedures combining chemical analysis and specific bioassays. This approach was focused on health and environmentally relevant compounds and based on bioaccumulation evaluation, to find a correlation between organochlorinated compounds, chlorobiophenyls congener and polycyclic aromatic hidrocarbons concentrations in sediments and co-generic sea anemones from three different climatic scenarios. The sea anemones species *Anemonia sargassensis, Anemonia sulcata, Actinia bermudensis, Actinia equina, Bunodossoma caissarum* and *Bunodossoma canjicum* was chosen because they might possibly be effective pollution indicators and early warning signilizer. They are very common and live in marine coastal environments which are continually exposed to garbage dumping, untreated sewage inflow, land and river runoff, atmospheric fallout from heavy traffic and various small-scale industries. In this work we observed the potential of sea anemones to provide valuable new insights into ecotoxicological answers and to make the functional link between environmental effects and human health level disturbances. Data for bioaccumulation on sea anemones were obtained on tropoical, subtropical and temperate ecosystem. Results indicated the involvement of this species in the bioaccumulation of a large spectrum of POPs, primary organochlorine contaminants such as PCBs, DDTs, and other pesticides, and PAHs such as Pyrene, Benzoantracene, Benzopyrene and other. The consequences of these large spectrum of contaminant accumulation could be accuracy in future laboratorial assessment studies, using biochemical tools.

WE295
Dioxins and PCBs in eel and Chinese mitten crabs in the Rhine-Meuse estuary
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Although concentrations of dioxins and PCBs in eel (*Anguilla anguilla*) from the Rhine-Meuse estuary have dropped markedly since the 1970s and 1980s, current levels in the Dutch part of these rivers are still above European consumption standards. During the 1980's the fishery and consumption of eel was restricted and since 2011 the fishery and trade of eel and Chinese mitten crab (*Eriocheir sinensis*) has been forbidden in most sections of the large rivers and connected waterways in the Netherlands. *E. sinensis* is an invasive species with high densities in the estuarine areas and the fisheries during the autumn months have increased during the last decade. The present study was focused on temporal variation in dioxin and PCB concentrations in different size classes of eel and Chinese mitten crabs at ten locations in the restricted area. Juvenile eel (28-32 cm) from an exposed location were translocated to a clean site (Berkenwoude) and the effect on the dioxin and PCB concentrations were followed. For both eel and Chinese mitten crabs a considerable variation was observed between locations, with in general relatively lower concentrations at the more coastal (Maasvlakte, Haringvliet). High concentrations were found in the hepatopancreas (or brown meat) of the Chinese mitten crabs; concentrations in the white muscle meat of legs, claws and body were low and well below the European consumption standards at all locations. In eel, concentrations increased markedly with size classes; for the small eel (28-32 cm) concentrations were below or around the consumption standard. In the translocation experiment, a marked decrease (almost 50%) was noted after 1 year and attributed to growth dilution and probable biotransformation of some congeners with lower chlorination. The results will be discussed in relation to the perspective of sustainable management and fisheries on eel and Chinese mitten crabs in the Netherlands.

WE296
Pendimethalin - Refined Characterization of Persistence, Bioaccumulation and Toxicity under Regulation EC 1107/2009
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The persistence, bioaccumulation and toxicity (PBT) of the herbicide pendimethalin is assessed in more detail using results from GLP guideline studies and open literature as single isolated results indicate values at or above the trigger given in EC 1107/2009. Regarding persistence in soil, geometric mean DT_{50} values from laboratory studies as well as field studies were in the range of 72-83 d (normalized and non-normalized) thus below the P-criterion of 120 d. In field soil accumulation studies no accumulation was observed after several years of annual application providing strong evidence that pendimethalin is not persistent in soil. In freshwater, aqueous photolysis is the main degradation process of pendimethalin with DT_{50} values less than the P-criterion value of 40 d for water. Moreover, pendimethalin rapidly partitions from water to sediment with a geomean $DisT_{50}$ < 1 d. Thus, sediment is the relevant compartment of degradation for pendimethalin; DT_{50} values for total water/sediment system ranged from 4 to 103 d, (geomean 20 d), which is below the P-trigger value of 120 d for sediment. Aquatic mesocosm studies confirm the results of the water/sediment laboratory studies. Hence, it can be concluded that pendimethalin is not persistent in soil, water and sediment as specified in Annex II to Regulation EC 1107/2009. Standard BCF studies reflect the potential for uptake in fish following constant, long-term exposure. The geomean for BCF out of 3 studies is 1518, i.e. below the trigger of 2000. Considering the very rapid dissipation of the substance from water (DisT50 < 1 d) long term exposure is an unrealistic situation while uptake via food may be more relevant. BMF studies have been conducted (geomean 0.065) and are well below the threshold of concern for bioaccumulation (BMF = 1) demonstrating that biomagnification through diet in fish does not occur. All lines of evidence (i.e. laboratory studies, higher tier studies, literature data) indicate only moderate potential for bioconcentration and no potential for biomagnification. Therefore, pendimethalin cannot be classified as bioaccumulative under EC 1107/2009. The EC_{10} value (geoman 11 μg/L) for primary producers as the most sensitive group is greater than the threshold of 10 µg/L indicated as T criterion by EC 1107/2009. Based on the overall information and weight of evidence it can be concluded that pendimethalin does not fulfil the PBT criteria.

WE297
Persistence of pharmaceuticals measured in two Swedish lakes by chemical benchmarking
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Persistence is one of the core criteria for exposure and hazard assessment. It is normally expressed as a degradation half-life in a single medium such as air, water or sediment. There is currently no standard method to assess the persistence of chemicals in real environmental systems. We recently proposed a method that exploits chemical benchmarking to measure the persistence of chemicals in real lakes [1]. Our benchmarking method can be applied in lakes that do not have pronounced stratification, have well-characterized water residence times, and where there is a single major source that releases benchmark and test chemicals at constant concentration ratios to the lake, such as the effluent of a single sewage treatment plant (STP). We identified two Swedish lakes (Lake Boren and Lake Norra Bergundsjön) to test the method. Water samples were taken at the beginning of June 2013 at the inflow and the outflow of both lakes, and weekly flow-proportional effluent samples were collected for nine weeks at the STPs on each lake to assess variability in concentration ratios of benchmark and test substances. A self-packed SPE cartridge (500 mg) consisting of three sorbents (Oasis HLB, MAX and Isolute ENV+) with a weight ratio of 1.9:1:1.2 was used to enrich the target analytes including twelve pharmaceuticals, one personal care product (climbazole) and one artificial sweetener (acesfulfame potassium). The analytes were analyzed by UHPLC/QqQ-MS. Mass balances of five compounds (carbamazepine, sulfamethoxazole, fluconazole, diatrizoic acid and acesfulfame K) indicated that they are suitable as benchmark chemicals. In Lake Norra Bergundsjön the persistence of test chemicals such as gemfibrozil, diclofenc and bezafibrate could be estimated from the concentration ratio between benchmark and test chemicals at the point of emission from the STP and the outlet of the lake. In Lake Boren the inflowing water, and not the STP, was the dominant source of all of

the pharmaceuticals that were identified as potential benchmarks. Therefore the persistence of test chemicals in Lake Boren was calculated with a modified version of the benchmarking approach used in Lake Norra Bergundsjön. The estimated persistence of the test chemicals in the two lake systems will be compared and discussed. [1]H. Zou, M. MacLeod, and M. S. McLachlan, Chemosphere, vol. 95, pp. 301–9, Jan. 2014.

WE298

Improvements on the Brazilian Antifouling Research Network (BARN)

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The use of antifouling coatings has caused many environmental problems during the last decades in coastal areas under the influence of maritime activities. However, the banning of tributyltin(TBT)-based antifouling paints issued by International Maritime Organization from September 2008, and its replacement by less persistent biocides, has led regulators and researchers around the world to consider the environmental issues related to TBT as an overcame problem. High TBT and imposex levels are, however, still being detected in many South American coastal areas. Based on this scenario, the Brazilian Antifouling Research Network (BARN) was structured in order to study, either spatially and temporally, the occurrence, impacts and processes associated to antifouling biocides along the Brazilian coastal shore. The BARN incorporated researchers from 12 Universities and has analytical capacity in three different laboratories to analyze antifouling biocides in environmental samples. Thus, concentrations of butyltin compounds, irgarol 1051, diuron, sea nine (DCOIT), busan (TCMTB), chlorotalonil and dichlofluanid are being determined in environmental samples from at least 24 of the main harbor areas of Brazil. At the same time, imposex levels in gastropod mollusks (biological effect of TBT contamination) is been thoroughly assessed. The preliminary results have suggested that 3 different scenarios are found along the Brazilian coast for TBT contamination. A fresh TBT input was clearly seen along many areas from the Northern and Northeastern coast of Brazil, which is less industrialized and where environmental issues are less controlled. In the Southeastern region, most populated and industrialized area, some improvements in the TBT and imposex levels were seen, although fresh TBT inputs were still detected as well. Finally, a significant and consistent decrease in the TBT and imposex levels was characteristic from the coastal areas of Southern Brazil. Based on that, a management plan is been proposed in order to reduce the environmental issues related to the use of antifouling paints in Brazil.

WE299

Screening-level risk assessment of active ingredients in cleaning products based on the usage patterns of consumers

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After chemical accident by fatal misuse of humidifier disinfectant in Korea, household chemical products such as cleaning products, wet tissue, air freshener, deodorant, etc., have been screened by consumer health risk based on consumers use pattern in Korea. In particularly, cleaning products were registered amount to 1300 brands and covered about 65% of total brand number of household chemical products in Korea. Nevertheless, consumer exposure to cleaning agents did little research, and lack of relevant factors for risk assessment. Cleaning products are known to consist of different group of active ingredients such as surfactants, preservatives and solvents. However, list of active ingredients of the registered cleaning products were not unknown in Korea. So in this study, for the identification of chemical and cleaning products of concern, all of registered cleaning products are screened against their active ingredients reported by producers in Korea and other foreign database such as Household Product Database in USA. All of registered cleaning products were classified as several product types according to the on-line and off-line market survey, and their exposure scenarios were identified according to the activity of consumers during preparation/loading, using, and after using periods. In this study, exposure factors such as use frequency/period/time and the amount of product use were also investigated by on-line consumer survey. In the first step, assuming that active ingredients were used in the cleaning products, product types and active ingredients were screened by the consumer product risk assessment, where the exposure levels for each active ingredient for every types of cleaning products to consumers were compared to toxicological acceptable levels. At the results, the differences between domestic and foreign exposure factors in same purpose products were clearly confirmed. This study suggests the importance of domestic exposure factors in safety management for cleaning agents through risk assessment. Finally, chemicals and product types of concern was confirmed. According the results of screening-level of risk assessment, the pairs of chemical-product type will be assessed by the second steps of risk assessment, which is a sort of refined consumer product risk assessment based on the measured contents of active ingredients.

WE300

Source apportionment and spatial distribution of polycyclic aromatic hydrocarbons (PAHs) in Swart River, Cape Town

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This study investigated levels and source input of selected polycyclic aromatic hydrocarbons (PAHs) in Swart River in Cape Town, South Africa. The PAHs in the water and sediment samples were extracted using liquid-liquid extraction and microwave assisted extraction/liquid-liquid extraction procedures respectively. PAHs extracts were cleaned up using solid phase extraction on neutral/basic/acidic/neutral silica column. The cleaned extracts were separated and quantified using gas chromatography coupled with flame ionization detection (GC-FID). Benzo[a]pyrene (B[a]P), benzo[a]pyrene (B[a]A), benzo[k]fluoranthene (B[k]F) and indeno[123-cd]pyrene (IP) ranged < 0.001 – 3.72; 1.52 – 2.25; 1.42 – 5.04; and 1.94 – 5.92 μg/L respectively in water, and 1.05 – 4.82, 2.57 – 4.63, 1.98 – 5.74 and 3.74 – 7.64 μg/kg respectively in Swart River sediments. Levels of the PAHs were in the order IP > B[k]F > B[a]A > B[a]P Results from principal analysis (PCA) of PAHs sediment accumulation factors, showed that, there were variations in the levels of the PAHs detected in sample clusters derived from different sampling stations along the river. This suggests that the neural of the different sources of water networks delivering their contents into the Swart River may have varied contribution of PAHs input.

WE301

Consideration of environmental substance stock dynamics for informing decision-making on persistent substances in the environment.

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In the context of prioritising regulatory action of substances, dynamic (time based) modelling greatly assists in the assessment of which substances and their uses will lead to impacts that will persist. In addition, in the context of socio-economic analysis it assists in the setting of relevant timescales for the assessment of impacts, so that damage costs of persistent substances can be compared to the benefits of continued use of the substance. Regulatory decision making on substances that may have environmental impacts requires consideration of how long substances may stay in the environment and exert undesirable impacts. A key point in regulatory decision making on limiting the uses of substances (such as in the process of Authorisation and Restriction under the REACH Regulation) is the analysis of the potential impacts caused by continued use of a substance verses the benefit that continued use may bring. In the assessment of environmental exposure, a steady state of predicted environmental concentration is calculated on the basis of sustained input from releases to the environment from the use of a substance. For persistent substances there will be a load or stock of substance that remains in the environment even if all emissions cease. Therefore, there is the potential for impacts to continue for some time after a regulatory action has been taken. Introduction of a time-based component to environmental exposure modelling has been developed. Using this model allows scenarios to be tested in which specific uses of a substance can be limited or added and the outcomes in terms of the concentrations, and total amounts in the environment over time and at different scale to be estimated. It is then possible to use this as a basis for understanding the potential impacts of the substance in time and space. Consideration was made of a small number of different substances that all fulfil the criteria for persistent, bioaccumulative and toxic/very persistent and very bioaccumulative (PBT/vPvB) and the differences that environmental degradation over time makes to the stock of substance in the environment. We also consider how that can be then be used to assess impacts within the regulatory and socioeconomic context.

WE302

Screening and prioritization of chemicals in the context of REACH: the cumulative PBT Index model implemented in QSARINS

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The limited availability of comprehensive data for Persistence, Bioaccumulation and Toxicity (PBT) of chemicals is a serious hindrance in the assignment of any chemical to the category of PBTs or vPvB, chemicals that require an authorization in REACH for their use and additionally plans for safer alternatives. In the context of screening and prioritization tools for PBT-assessment, explicitly required in REACH regulation, the cumulative PBT Index model, implemented in QSARINS (QSAR-INSUBRIA), a new software for the development and validation of multiple linear regression (MLR) Quantitative Structure-Activity Relationship (QSAR) models by Genetic Algorithm-Ordinary least Squares (GA-OLS), offers a new holistic approach to identify chemicals with cumulative PBT properties, directly from their molecular structure. In this study the Insubria-PBT Index in QSARINS has been applied to screen and prioritize big datasets containing large variety of chemicals of environmental concern with heterogeneous molecular structure. Particular attention has been taken in the study and definition of the Applicability Domain of the model, using different approaches such as Descriptors Range, Leverage, and Principal Component Analysis (PCA) of the modeling molecular descriptors, in order to propose reliable predictions. The results of this study, which is based only on the molecular structure features and is not dependent on single threshold values for P, B and T, have been compared with those obtained by the on-line US-EPA PBT profiler and in other published screening studies. A good agreement among various approaches has been found, supporting the need of a consensus approach in these identification studies. The discrepancies have been highlighted and commented. Moreover, a priority list containing the most dangerous compounds has been drafted identifying the common structural features among the potential PBT chemicals. The PBT-Index implemented in QSARINS has demonstrated to be a practical and reliable solution for PBT-identification, immediately from the chemical structure, thus even before the synthesis, in a benign by design approach.

WE303

Literature review in toxicological research and chemical risk assessment: the state of the science

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Objective: Literature review techniques have changed markedly in the last 30 years in medicine, providing a transparent and accessible evidence base for the resolution of controversy and uncertainty about the efficacy of interventions in medicine. We examine the extent to which these techniques have been taken up in literature review in the toxicological sciences. **Method**: We develop a model of the ideal literature review process based on best practice in evidence-based medicine. From this, we derive what we believe to be the first toolkit for evaluating the quality of literature reviews of toxicological research. We apply this toolkit to a representative sample of literature reviews of bisphenol A (BPA). **Results**: Very few literature reviews of toxicological research are being conducted according to scientific standards. Analysis of the European Food Safety Authority's 2010 and draft 2013 Scientific Opinions on BPA shows serious shortcomings including: lack of clarity in objective; potential sampling bias resulting from the search methods used; lack of clarity or consistency in how studies were evaluated for methodological quality; inadequate declarations of interest; and discrepencies between findings reported in the body of the reviews and those presented in summary texts. **Discussion**: The state of the science of reviews of toxicological research is reminiscent of findings in the 1980s in medicine: they are often subjective and scientifically unsound; strategies for identifying and selecting information to prevent sampling and selection bias in the evidence base are rarely defined; evidence of toxicity is reviewed haphazardly without sufficient attention to systematic assessment of either its quality or directness. In these circumstances, reliable and accurate summarisation of evidence seems virtually impossible. In case of disagreement, the lack of clearly documented methodology (the keystone of the scientific method) makes it extremely arduous, if not impossible, to determine which of two conflicting opinions is most likely to be correct. **Conclusion**: We recommend the development and application of systematic review techniques to the literature review process in chemical risk assessment. This will provide a transparent and accessible evidence base on which to base policy decisions, providing the necessary scientific contribution to resolving controversy and societal uncertainty about chemical safety.

WE304

Feedback from an Authorization Dossier: Research of Alternatives, the Cornerstone of the Process.

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We have recently performed a socio economic analysis (SEA) of the substitution of a CMR/PBT substance under Biocide regulation. Because no guideline existed under biocide regulation, we based our strategy on those existing under REACH. As it was one the first attend, we faced several questions and one of the most problematic was about the alternatives. Indeed, we showed that the research of alternatives phase is the cornerstone of an authorization dossier. That phase would define the impacts both in human health and environment but also on the economic impact. The alternatives research could lead to a wide range of economic impacts. Therefore, from our point of view, the need of guidance should be first on how to determine what a good alternative is. Several questions remained opened and could significantly affect the scope of the socio economic analysis. One is the *time* scope for searching potential alternatives. Should the applicant only consider the alternative available at the *t* time of the authorization, or also have to include the alternatives which could fit its needs in the near future? Another question refers to the completeness of the alternatives' impact assessment. An alternative requires more or less adjustment of the production process or of the regulatory certificates of a given product. Technological and economic knowledge of the different alternative are heterogeneous, depending on the already purchasing behavior of the firm. Should the applicant describes and evaluate all the alternatives with the same level of completeness? We showed that the choice of alternatives is not only a technical question. The regulatory constraint on alternatives choices determined the scope (and so the cost) of the SEA. It also fixed the level of the adjustment effort of the firm implicitly asked by the regulator (in terms of R&D process and/or transformation of ongoing production process). We will propose some safe-harbor to better frame the AoA phase of the SEA, including a two-step process that allow making a primary filter in a first step, before undertaking a more detailed analysis in a second step.

Environmental OMICs: high-throughput strategies to decipher mechanism of response to stressors (P)

WE306

Development of native sediment ecotoxicity species Glyptotendipes tokunagai using comparative ecotoxicogenomics approach with Chironomus riparius

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Insect species are suitable test organism for risk assessment investigation of the environmental contaminants, as these are the largest group of animals, and ecologically one of the most important groups. In particular, *Chironomus riparius* is widely used as global ecological model species of ecotoxicological studies. Recently, transcriptome database of *C. riparius* were established in our laboratory, and a number of stress response genes were identified from this database. However, since, *C. riparius* is not Korean native species, so it limits application for local specific ecological risk assessment. The *Glyptotendipes tokunagai* is a dominant aquatic insect species that exists widely in East Asia including Korea and appears frequently in organic rich urban streams. However, eotoxicological studies of *G. tokunagai* have so far been hampered by limited knowledge of biomarkers of these species. Both *C. riparius* and *G. tokunagai* are the tribe *Chironomini*, and *C. riparius* belongs to the Genus *Chironomus*, while *G. tokunagai* belongs to the genus *Glyptotendipes*. These two genus are shown highly phylogenetic similarity in a number of studies. Therefore, in this study, in order to develop *G. tokunagai* as a native specific ecotoxicological species in Korean ecological risk assessment, we investigated gene and protein level biomarkers in *G. tokunagai* through comparison with *C. riparius* omics information using ecotoxicogenomics approaches. Overall stress response genes and proteins expression results showed that molecular biomarkers in response to environmental contaminants using *C. riparius* information could have a considerable potential in *G. tokunagai*. This study can contribute to develop *G. tokunagai* as native sediment ecotoxicity species.

Reduction, Replacement, and Refinement (3Rs): Animal alternative approaches in ecotoxicology and risk assessment (P)

TH001

Comparative vitellogenin expression in two alternative fish models using 17α-Ethynylestradiol

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The use of alternative models is currently receiving wide attention to support the ecotoxicological evaluation of the potential risk of aquatic pollutants, with an overall aim to reduce and ultimately, replace live fish testing. Current *in vitro* systems such as 2-D monolayer cultures and freshly isolated suspension cultures allow for rapid screening of pollutants, yet there loss of functionality over time and reduced cell-cell interactions limit their suitability as a direct surrogate for *in vivo* systems. Moreover, few studies exist on the direct comparison between *in vitro* and *in vivo* systems and their relative sensitivities to chemical exposure. Here we report a study that compared the expression profiles of the vitellogenin gene (vtg) in a rainbow trout (*Oncorhynchus mykiss*) 3-D liver spheroid (*in vitro*) and zebrafish *(Danio rerio*) larvae (*in vivo)* model, after exposure to environmentally relevant levels of the endogenous estrogen 17α-Ethynylestradiol (EE2). Following optimisation of RNA extraction and reverse transcription for the spheroids and zebrafish larvae, these two models were exposed to a range of EE2 concentration (0.01 – 1 μg L^{-1}) to determine NOEC and LOEC values. A time-course exposure was then used to profile vtg expression using qPCR. Suitable housekeeping genes (i.e. 18S rRNA for spheroids and β-actin for the larvae) for each system were also fully validated. Both models demonstrated up-regulation of vtg after exposure to EE2. Whilst significant level of vtg expression was not detected in either model after 24 h EE2 exposure, this was detected in liver spheroids after 72 h exposure (0.1 μg L^{-1}) compared to Zf larvae which showed expression after 48 h exposure (0.5 μg L^{-1}). These preliminary findings suggest similar sensitivities in Zf larvae

and liver spheroids to EE2 exposure. Overall, the results suggest that trout liver spheroids may be useful for gene expression analysis and as an alternative *in vitro* model for metabolism, bioaccumulation and toxicity studies. Keywords: Alternatives, vitellogenin, spheroids, zebrafish Abstract type: Poster

TH002

Molecular and physiological effects in zebrafish developing embryos exposed to binary mixtures of herbicides suspected of endocrine disruption

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The identification of endocrine disruption chemicals (EDCs) and their mode of action (MOA) is a major scientific challenge in experimental toxicology. Complexity increases when assessing joint action of EDCs with the same or different MOA. Despite *in vivo* reproduction tests with fish are a mandatory component in the base-set of data for EDC screening, regulators also promote reduction of vertebrate animal testing or even replacement by alternative methods. Among the existing alternative methods to replace animal testing for EDCs screening, the fish embryo test (FET) complemented with toxicogenomic tools arises as a promising alternative. In this context, the aim of the present work is to evaluate the physiological endpoints (e.g. malformations) and characterize the molecular changes induced in zebrafish developing embryos exposed to two herbicides suspected of endocrine disruption. In order to achieve that, the embryos were exposed to linuron and s-metolachlor (single and in binary mixtures) at concentrations above the EC_{10} of mortality and malformations. Embryos were checked for mortality and malformations during the 96h exposure. At the end of exposure embryos were snap-frozen for RNA extraction. A suite of genes were chosen for expression levels' assessment by quantitative real-time PCR (qPCR) during early development of zebrafish due to their importance in reproduction of teleost fish as well as potential targets of EDCs. Alterations on the apical endpoints and on gene expression were evaluated and discussed. With this we intend to unravel MOA of the tested substances and predictive endocrine disruption biomarkers useful for the development of FET as an alternative method for EDC screening.

TH003

Development of a microfluidic bioreactor for culture and analysis of the piscine intestinal cell line RTgutGC

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Background. The epithelia of fish skin, gills and the intestine are gate keepers that regulate the interaction of the organism with its surrounding environment. Chemicals present in the aquatic environment first have to pass these barriers before they can cause harm to other organs. With the aim to improve our understanding of fish physiology and mechanisms of toxicant mode of action in fish we develop a "fish-gut-on-chip" based on a continuous cell line from rainbow trout (*Onchorynchus mykiss*). **Methods**. The term "fish-gut-on-chip" refers to a bioreactor where two flow channels are separated by an ultrathin nanoporous membrane, which serves as support for the rainbow trout intestinal cell line, RTgutGC. The basic concept of the design is to closely mimic the cellular microenvironment as found in the organism and to allow for more realistic exposure and transport scenarios. **Results**. The ultrathin membrane, which builds the core of the microfluidic bioreactor, is composed of anodized aluminum oxide and realized in a silicon chip using microfabrication technology. The membrane is characterized by a thickness of only 1 μm, a high porosity and a tunable pore size of 30-130 nm. Cells can be grown on both sides of the membrane, making it suitable for co-culture studies. A syringe pump allows for accurate flow conditions within the two microchannels of the bioreactor. Additionally, the bioreactor is equipped with electrodes for on-line impedance spectroscopy. **Outlook**. In future studies, we aim to co-culture the intestinal cell line RTgutGC with the fibroblast-like cell line RTHDF as supportive cells. We will examine the benefits of the co-culture set-up according to barrier properties and analyze the uptake and metabolism of a model toxicant by the *in vitro* barrier. **Keywords.** organs-on-chips, fish cell culture, microfluidics, impedance spectroscopy

TH004

Rainbow trout gut cell line (RTgutGC) characterization as a model for fish intestinal epithelia

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The gut of fish is a multifunctional organ not only involved in absorption of nutrients but also in salt and water homeostasis, gas exchanges, acid-base balance, nitrogen metabolism and endocrine/neuroendocrine functions. Our knowledge and understanding of this organ so far comes from *in vivo* studies and *ex vivo* studies such as the gut sac preparation. The development of an *in vitro* model for the fish gut has been a desire of the fish physiology and toxicology scientific community for a long time. A model of the polarised intestinal epithelia is of pivotal importance to further our knowledge of this tissue at the cellular and molecular level. Recently an intestinal cell line (RTgutGC) has been described[1]. In this study we are characterizing this cell line with particular emphasis on its suitability as a model of polarised intestinal epithelia. When grown on transwell inserts in culture plates, RTgutGC cells develop a trans-epithelia electrical resistance comparable to *in vivo* measured values[2], express the tight junction protein (ZO-1) and show clear formation of desmosomes as shown by transmission electron microscopy. Moreover, confocal images in the z dimension show evidence of polarization with a morphology, previously shown for the polarized fish gill[3], such as distinct apical/basolateral actin staining. Other important proteins for the intestinal function present in RTgutGC cells include Villin1, Na/K-ATPase, V-ATPase and CYP1A. References (1) Kawano, a., Haiduk, C., Schirmer, K., Hanner, R., Lee, L. E. J., Dixon, B., and Bols, N. C. (2011) Development of a rainbow trout intestinal epithelial cell line and its response to lipopolysaccharide. *Aquac. Nutr. 17*, e241–e252. (2) Sundell, K., Jutfelt, F., Olsen, R., Sandblom, E., Hansen, T., and Bjornsson, B. T. (2003) Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr – smolt transformation of Atlantic salmon , Salmo salar. *Aquaculture 222*, 265–285. (3) Sandbichler, A. M., Egg, M., Schwerte, T., and Pelster, B. (2011) Claudin 28b and F-actin are involved in rainbow trout gill pavement cell tight junction remodeling under osmotic stress. *J. Exp. Biol. 214*, 1473–87.

TH005

Fish cell lines in 3 dimensional (3D) cultures in ecotoxicological studies: is there oxygen in the middle?

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In mammalian research, three-dimensional (3D) *in vitro* culture techniques, are a well-established model considered to be exhibiting micro environments which are close to that of the *in vivo* conditions. In parallel, the fish liver spheroid model is now being considered for environmental applications in line with the 3Rs approach (reduction, replacement, and refinement). The limitations of fish spheroids originating from cells of other organs have not yet been systematically assessed. A stumbling block to the widespread use of this technique in fish ecotoxicology is the lack of information regarding the morphology and physical micro-environment of spheroids. It is not known whether the spheroids, formed from fish cells, form the same micro-environment as tumour models or whether they are more representative of the *in vivo* tissue they are formed from. Oxygen gradients across the micro-structure are believed to play a very important role in driving xenobiotic metabolism. It is therefore important to know whether the growing spheroids have a homogenous supply of oxygen, which would have a considerable impact on the metabolism of xenobiotics, and provide key information as to the likely applicability of the model. For the first time, the oxygen saturation level within spheroids formed from the rainbow trout gonad cell line RTG-2 was measured using the novel application of Electron Paramagnetic Resonance (EPR). Spheroids were grown in a range of sizes from 100μm to 800 μm, with the EPR oxygen sensitive probe lithium phthalocyanine embedded at the core. Initial findings indicate that for smaller spheroids (as we currently use), percentage oxygen saturation was approximately 88% in the 100-300μm size range up to 7 days post formation. Interestingly over a 14 day period, oxygen saturation levels halved from day 7 to day 14 in these smaller spheroids which was not replicated in the larger spheroids. Larger spheroids (500-800 μm) had a lower initial oxygen saturation level (21%) when sampled on day 7, with a complete loss of cell viability observed at 14 days with these big gonadal RTG-2 spheroids. Overall, the oxygen measurements within this spheroid model suggest that fish spheroids have a homogenous supply of oxygen at smaller size ranges at least for the first week. As they mature, it appears that an oxygen gradient is formed that may mimic the conditions identified in mammalian tissue, but are poorly understood in fish.

TH006

Waiving chronic fish tests: possible use of acute-to-chronic relationships and interspecies correlations

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EU chemicals policy (industrial chemicals, biocides, etc) encourages the use of all available information for hazard and risk assessment before new tests on vertebrates are proposed or conducted. In this context, the usefulness of fish to acute toxicity relationships and interspecies extrapolations for waiving chronic fish tests have been explored. Data were extracted from the US EPA ecotox Database (n=86) and the OECD QSAR Toolbox (n=73) and analysed, both as a whole dataset and classified by their mode of action (MOA). We analysed: 1) *Daphnia* (acute and chronic) to fish chronic toxicity relationships, and 2) fish acute to chronic toxicity relationships. Our first analysis indicates that long-term, or even short-term, testing with *Daphnia* will usually protect against fish chronic effects. In particular, linear regressions provide a reliable basis for extrapolating from acute or chronic *Daphnia* data to predict fish chronic toxicity for chemicals with Verhaar MOAs 1 and 3 (i.e. non-polar narcotics and chemicals with non-specific reactivity). However, the relationships are less reliable for chemicals with MOAs 2 and 5 (i.e. polar narcotics and miscellaneous chemicals). Regarding MOA 4 chemicals (i.e. those having specific reactivity) there were insufficient data for linear regression analysis. In our second analysis, the relationship for all chemicals (irrespective of the MOA) obtained between fish acute and fish chronic toxicity data is much better than the one obtained with *Daphnia* data. The relationship for MOAs 1 and 3 chemicals are nearly the same as those obtained with *Daphnia* data, whereas, in the case of MOAs 2 and 5 chemicals, fish acute toxicity data predict fish chronic toxicity better than *Daphnia* acute toxicity data. Our preliminary results suggest that when fish acute toxicity data are availabel they could be used to predict fish chronic toxicity; however, if fish acute data are not available, then daphnia data could be used, as long as the predictive accuracy for differents MOAs is kept in mind. Our findings show the potential of data-based approaches; however, the data collection and analysis should now be extended to more chemicals. Acknowledgement: Part of this work was carried out under the terms of a service level agreement between ECHA and the JRC. Disclaimer: The opinions expressed herein are those of the authors and do not necessarily reflect the official views of the European Commission.

TH007

Systematic approach to investigate outliers of the fish embryo test to increase its predictive capacity and applicability domain for acute fish toxicity and beyond

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Fish embryos represent an accepted alternative to animal experiments. In contrast to cellular models they represent a complex system that is considered to more closely resemble an adult, differentiated organism. Various regulatory applications in environmental and human hazard/risk assessment for the registration of industrial chemicals, plant protection products, biocides, pharmaceuticals, cosmetics and feed additives are discussed – ranging from prediction of acute and chronic fish toxicity, endocrine disruption, organ toxicity, neurotoxicity, human acute toxicity to teratogenicity. The present study aimed at identifying and analyzing outliers of the Fish Embryo Test (FET) – acute fish toxicity (AFT) test regressions making use of a previously established database of fish embryo toxicity data for about 640 compounds. Outliers were identified by box blot analysis and an arbitrary deviation threshold of 50fold from the regressions of FET/AFT. We confirmed the high correlation of FET/AFT but identified 35 potential FET outliers out of 172 compounds. Outliers were subjected to an evaluation of the potential role of physico-chemical properties, mode of action or metabolic activation. The outlier analysis has revealed enrichment of neurotoxic compounds indicating that this mode of action may not primarily result in mortality of fish embryos at concentrations in which acute fish toxicity is observed. To increase the sensitivity of fish embryos, indicators of neurotoxicity (behavioral assays which quantify changes of embryonic movements) and other sublethal, mode of action related endpoints have been considered. By this systematic study as well as identification and understanding of outliers we are aiming to better define or extend the application domain, improve, reduce uncertainty and finally improve the predictive capacity.

TH008

Bioconcentration of Human Pharmaceuticals: How useful is the trigger Kow for the performance of a fish study for the Environmental Risk Assessment?

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The potential risk of human pharmaceuticals to the environment has to be determined in an environmental risk assessment (ERA), e.g. according to the EMA Guideline EMEA/CHMP/SWP/4447/00. In this guideline, the octanol/water partition coefficient (Kow) is used as indicator for the potential of bioaccumulation in aquatic organisms. If the trigger of log Kow > 3 is exceeded, the bioconcentration factor (BCF) should be considered. Usually, a fish bioconcentration study according to OECD test guideline 305 is performed in such case. However, this study requires a large number of animals. The measured BCFs in fish studies with human pharmaceuticals are often rather low compared to the theoretical BCFs based on model predictions calculated from the Kow and by QSARs. Examples will be given in this presentation. This is not uncommon, since many pharmaceuticals are ionisable chemicals, where the Kow is a weak indicator for the bioaccumulation potential due to the pH-dependent dissociation of the molecule. Additionally, pharmaceuticals are often metabolised and/or quickly depurated in vertebrates including fish. Hence, the question arises how useful the trigger of log Kow > 3 is for the performance of a fish bioconcentration study with human pharmaceuticals. In ERA the BCF is in practice mainly used for prove of the B-criterion (bioaccumulation) in the PBT assessment. However, for the ERA of human pharmaceuticals also a water-sediment transformation study and long-term toxicity studies with fish, daphnia and algae are required in most cases, so that data to the P-criterion (persistence) and the T-criterion (toxicity) are available anyway. The trigger value of log Kow > 3 for the performance of a fish bioconcentration study should therefore be discussed. Alternatives will be suggested to strengthen animal welfare considerations by inclusion of the Integrated Testing Strategy (ITS) under REACH (ECHA Guidance R.7c) also in the environmental risk assessment of human pharmaceuticals.

TH009

A thermodynamically driven QSAR model to predict aquatic toxicity for mixtures

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Abstract: Reliable estimations for the aquatic toxicity of mixtures has been of high concern for many years. To achieve this, the current OECD Guidance No. 23[1] on difficult substances and mixtures advocates use of the water-accommodated fraction (WAF) method (i.e. testing the constituents together at specific loading concentrations). Implementation of the WAF method costs time and money. More importantly, the concentration of each constituent in the WAF is not the same as the original fraction within the mixture. This is due to the thermodynamic influence of the constituents on each other. The Classification, Labelling, Packaging (CLP) regulation[2] based on the GHS[3] specifies use of a calculation method based on the fraction and aquatic toxicity of each constituent. Nevertheless, this usually leads to an overestimation of the aquatic toxicity of the mixtures often resulting in an over-classification of the substance. Recently, a new iSafeRat® High Accuracy-QSAR (HA-QSAR) model[4] has been developed using a thermodynamic approach and additivity principle, which can determine the aquatic toxicity of mixtures with high precision provided the constituents follow a similar toxic mode of action. Based on the derived results, this work suggests that CLP/GHS calculations to estimate mixture toxicity can be improved. **Keywords:** WAF, aquatic toxicity, mixtures, *in silico* **References:** *1.* OECD guideline 23 [2000] Guidance document on aquatic toxicity testing of difficult substances and mixtures. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.3. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), United Nations Economic Commission for Europe, (Rev.4) 2011. iSafeRat® – *in Silico* Algorithms For Environmental Risk And Toxicity version 1.1.

TH010

Zebrafish embryotoxicity test (ZET): the importance of internal exposure analyses

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The transparent zebrafish (ZF) embryo is a promising alternative animal model for developmental toxicity. The ZF embryotoxicity test (ZET) is not regulated by current legislations on animal welfare, and is a cheap, high throughput approach, that enables continuous monitoring of development of a whole organism. Nevertheless, its true usefulness is determined by its predictability for animal and human reported data on teratogenicity, which is influenced in high degree by internal exposure of the ZF embryo. Since the uptake of test compound into the ZF embryo is crucial for a comprehensive evaluation and consequently classification of a compound, the concentration of test compound was determined, every 24 hours, in whole ZF embryos, dechorionated embryos, chorions, and hatched larvae. As a control, the compound concentration in the test solution was analyzed on a daily base. Because the chorion is an important barrier for penetration of compounds into the embryo, based on e.g. size and lipophilicy, these analyses were performed for compounds with a wide range in logP and molecular weights. Separate evaluation of test compound concentrations in embryo and chorion every 24 hours clarifies the role of the chorion as a barrier for compound uptake during the ZET. The value of logP and molecular weight as an indicator of compound uptake by the ZF embryo was also determined. Current studies only examined compound uptake by analyzing embryos that were dechorionated before exposure, or whole eggs. While the chorion is a lipid membrane, it is imaginable that lipophilic compounds could accumulate in this membrane, affecting the concentration found in whole eggs. This could give false positive results on internal exposure, while actual exposure of the embryo would be lower or absent. Consequently, analyzing the ZF egg as a whole

does not detect lack of internal exposure, which could lead to false negative results in the ZET. The separate analysis of embryo and chorion therefore not only clarifies the barrier function of the chorion during ZF embryo development of the ZET, but also gives information about the actual accumulation of test compound into the chorion, avoiding false negative results.

TH011

Challenges in bioanalytical water quality assessment: the polar, the volatiles, the unknowns – case study drinking water

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While drinking water disinfection is one of the major public health advances in the last century, one downside is that the disinfectants also react with organic and inorganic precursors to form potentially hazardous disinfection by-products (DBPs). After 35 years of research the major fraction of formed DBPs are not yet identified and hence, the bioanalytical evaluation of drinking water toxicity is a crucial complement to chemical analysis to evaluate drinking water quality. However, the high enrichment factors commonly required for bioanalytical assessment (up to 10,000-fold) entail the loss of a significant fraction of the total DBPs—in particular volatile and polar compounds, which are often overlooked in drinking water toxicity assessment. Here we explored different enrichment methods with the aim to minimise the loss of polar and volatile DBPs. Solid-phase extraction was the most efficient enrichment method for non-volatile DBPs with recoveries of total halogenated DBPs of ca. 70% compared to 13% for liquid-liquid extraction and 30% for freeze drying. Furthermore, we tested several purge & trap techniques for the extraction of the volatile fraction of total DBPs and will determine the portion of volatile DBPs compared to total DBPs. For the toxicological characterisation of the volatile fraction of total DBPs we adapted bioassays to minimise the loss of volatile DBPs during dosing and exposure to the samples. We successfully adapted three bacterial bioassays (Ames for mutagenicity, umuC for genotoxicity, Microtox for cytotoxicity) and two mammalian bioassays (AREc32 for activation of oxidative stress response and p53 induction as marker for genotoxicity) to a headspace-free design and could demonstrate an increase in apparent sensitivity towards volatile DBPs of up to three orders of magnitude. Finally, we evaluated drinking water samples using chemical analysis and bioassays. All detected DBPs will be toxicologically characterised as single compounds and in the mixtures in ratios found in the water samples to evaluate the toxicological relevance of the known DBPs compared to the unknown fraction. In conclusion this study highlights the importance of i) the adaptation of bioassays to a headspace-free setup to minimise the loss of volatile DBPs from drinking water samples and ii) the choice of extraction method as initial step that defines the types of chemicals, which can be targeted with bioassays.

TH012

Round-robin test of the RTgill-W1 cell line assay to predict fish acute toxicity

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Within the CEllSens project a strategy to predict fish acute lethality by industrial organic chemicals based on two model systems, fish cell lines and embryos of zebrafish, was developed. Upon establishment of the CEllSens chemical list, which was derived based on pre-defined criteria to cover chemicals with a wide range of physico-chemical properties, different modes of action and toxicity, the top 34 chemicals were investigated in a rainbow trout gill (RTgill-W1) cell line assay and the zebrafish embryo toxicity test. Concentrations causing 50% toxic effects ($EC_{50\ cell\ line}$ or $LC_{50\ embryo}$) were determined based on analytically confirmed exposure concentrations. Overall, a very good quantitative agreement was achieved for EC_{50} or LC_{50} values compared to LC_{50} from the acute fish lethality test, taken from the US EPA fathead minnow data base. The majority of test chemicals was within a 10-fold range from the line of unity of the alternatives vs. acute fish lethality tests if true exposure concentrations were accounted for. While the zebrafish embryo test has meanwhile been accepted as the OECD test guideline 236, the excellent performance of the RTgill-W1 cell line assay provided impetus to start bringing this potential animal replacement method to the same level of international acceptance. In addition to chemical regulation and potential application in effluent testing and product design, the cell line could also be implemented in the development of tiered approaches, e.g. along the lines of adverse outcome pathways to replace, reduce or refine chronic fish tests. Thus, with the support of CEFIC-LRI and UK NC3Rs, an Eawag-led round-robin test has been initiated involving six industrial and academic research laboratories from Europe and US. The overall goal is to test the robustness of the established methodology. In particular, we aim to evaluate the transferability of the RTgill-W1 cell line assay from the lead laboratory to other laboratories, and assess the within- and between-laboratory reproducibility. Participants are being trained in the lead laboratory to transfer the standard operating procedures (SOPs) and techniques. The reference chemical, 3,4-Dichloroaniline, is tested first by all laboratories. Then, five additional chemicals will be tested. Testing will include the cell viability analysis along with sampling for chemical analysis to confirm the exposure concentrations.

TH013

Fish cell lines : a screening tool for multiple stress scenarios. Interaction between fatty acid profile and heavy metals in rainbow trout hepatocytes (RTL-W1)

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In aquatic ecosystems, environmental conditions (e.g. temperature, food quality, water quantity...) are seldom optimal and change overtime. Though these variations may affect organisms' capacity to cope with chemical contamination such as heavy metals, they are rarely taken into account in classical ecotoxicological tests and most of the regulation on chemicals is based on single substance test performed under optimal conditions. Nevertheless, different environmental conditions (e.g. food quality) can modify the characteristics of an organism (e.g. nutritional status) which mayinfluence both the entrance of chemicals within this organism and its ability to cope with it. In particular, in fish, fatty acid composition can modulate membrane fluidity and thus influence contaminant uptake in the cells. Due to their different antioxidant properties, fatty acids are also likely to influence cells' sensitivity to oxidative stress induced by contaminants. Fish fatty acid profile is strongly influenced by nutrition and temperature, as a result, individuals from the same species may present strong differences in their fatty acid profile and may respond differently to contamination. Few studies have been exploring this hypothesis therefore an *in vitro* approach was set up to better explore the role of a wide range of fatty acids in fish response to contaminants such as metals. Fish hepatocytes from the same cell line (RTL-W1) but with up to 7 different fatty acid profiles were then obtained by enriching the growth medium withdifferent fatty acids of interest. Though these cells were from the same cell line; the modification of their fatty acid profile changed certain of their properties (e.g. membrane fluidity) and modified their response to increasing concentrations of mercury ($HgCl_2$ or CH_3HgCl) or cadmium ($CdCl_2$). For instance, cells enriched with the omega-3 fatty acid: alpha-linolenic acid showed a stronger resistance to organic mercury ($EC_{50}^{CH3HgCl} = 6.6\ \mu M$) than non-enriched cells ($EC_{50}^{CH3HgCl} = 2.9\mu M$) but a similar resistance to cadmium and inorganic mercury. The use of a fish cell line allowed screening the influence of various types of fatty acid profiles on heavy metals' sensitivity of fish. Based on these results, the multiple stress scenarios more likely to present a risk for the environment will beselected for further mechanistic (*in vitro*) and ecotoxicologic (*in vivo*) investigation.

TH014

What's the difference between a bird and a human? Comparative bioaccessibility for pharmaceuticals

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There is increasing interest from researchers, industry and policy makers in approaches to read-across from toxicological and pharmacological information on medicines, obtained from mammal models, to aquatic and terrestrial wildlife species. There are several issues associated with this approach, one of which is that it is usually assumed that the bioaccessibility of a pharmaceutical is equivalent across species. Bioaccessibility is the proportion of a compound that moves from the contaminated food/prey item into the digestive juice, and is predicted to vary between species due to differences in the physical and biochemical processes of digestion. Here, we propose that the use of Physiological Based Extraction Tests (Pbets), which have been developed to assess the bioaccessibility of heavy metals in a range of organisms, could be extended to pharmaceuticals. Pbets could be used to compare bioaccessibility between humans and non-target species and assist in extrapolating pharmacological/toxicological effects observed in humans to effects on wildlife. Here we demonstrate our method by comparing the bioaccessibility of fluoxetine in a human and a bird *in vitro* model. Fluoxetine is predicted to be ingested by birds foraging on invertebrates, such as earthworms, that have accumulated the compound from the environment (e.g. wastewater treatment works or sludge-amended soils). We exposed earthworms (*Eisenia fetida*) to fluoxetine spiked soil for 21 days. Earthworms were then inserted into a human Pbet model set up to simulate three feeding conditions. Bioaccessibility in birds was simulated using an avian Pbet and two different grit types (Ca or Si). The resulting digestive juices and residual solid material ("faeces") were extracted with solvent and analysed using High Performance Liquid Chromatography. We found that bioaccessibility was lower or equivalent to a human for birds in areas of Si grit due to the vast majority of fluoxetine being extracted in the gizzard. For birds in areas of Ca grit, bioaccessibility was higher than a fasted human, equivalent to an average human but lower than a recently fed human. Our methods could be used to support environmental risk assessments to support extrapolation from human effects data for pharmaceuticals to ecological impacts. This will not only provide time and cost

savings but also help reduce the number of *in-vivo* assessments performed in the environmental risk assessment process.

What do we know about the effects of multiple stressors and community responses on aquatic ecosystems? (P)

TH015

Cu accumulation and hepatic perturbations in yellow eels (Anguilla anguilla L.) experimentally exposed to multi-parametric stressor: metal (Cu), parasite (Anguillicola crassus) and osmotic challenge.

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Since the 1980s, the European eel (Anguilla Anguilla) population is in decline all over Europe. Consequently this species is now considered as endangered, outside safe biological limits. The cause of this decline is still unknown. Several factors can be evoked to explain eel collapse such as changes in oceanic circulation and food availability, overfishing, habitat disruption, chemical and pathogens contamination. During it growing continental phase, numerous young yellow eels stay in estuaries or in lagoons where they were exposed to pollution and parasite and simultaneously they were submitted to important variation in water salinity. To study the impact of such pluri-parametric stressor on young growing individual, little yellow eels were taken in Mediterranean lagoon of Canet, decontaminated during several weeks and then exposed to different stressors, alone or in combination, with environmentally realistic conditions: Cu, hypo- or hyper-osmotic challenge, infestation by Anguillicola crassus (nematode that lives in the eel swim bladders). The Cu bioaccumulation in muscle and in liver was followed during a 6 weeks experiment as well as histo-pathological perturbation and anti-oxidative biomarker of their liver. Additionally, the parasite infestation and the water salinity variation induced a clear modification of the Cu bioaccumulation observed with Cu alone.
Anti-oxidative biomarkers tested presented few variations in all the tested experimental conditions, but histological alterations were significant and contrasted among stressor. All stressed eels showed lysis and necrosis but Cu induced higher rates of hepatic fibrosis and nuclear alteration than parasite or salinity challenge. Infestation by *Anguillicola crassus* induced the highest immune response of the liver (melano-macrophage centers development and immune cell infiltration in the parenchyma) whereas liver of eel exposed to salinity variation presented important amount of lipid store in hepatocytes as huge lipid droplets. These alterations went simultaneously to a significant increase in eel mortality. Eels exposed to combination of stress presented highest rate of fish mortality in correlation to a clear increased of the hepatic alterations observed with stressor alone. It can be concluded that the combination of different stressors as it occur in littoral area can induce high rate of hepatic alterations and so may challenge the survivorship capacity of yellow eel.

TH016

Effects of hypoxia on the toxicity of copper to zebrafish (Danio rerio)

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Hypoxia is a global and increasingly important stressor in aquatic ecosystems, with major impacts on biodiversity. The causes of this global phenomenon are diverse and include climate change, which alters mixing and temperature of water bodies, and eutrophication, due to excess nutrient input from agriculture and sewage effluent. As a result, hypoxic waters often are also contaminated with multiple chemical pollutants. Despite this, little is known about the interactions between hypoxia and other stressors. Previous studies have demonstrated some remarkable alterations in the toxicity of some compounds when exposures occur under hypoxia. However, current knowledge spans only a few chemicals and species, and has incompletely investigated the life cycle of aquatic organisms, highlighting a major knowledge gap for evaluating the environmental impact of chemicals, where exposure often occurs under reduced oxygen. It is critical to investigate the interactions between these stressors in order to more effectively and objectively determine realistic safety thresholds for contaminants in aquatic ecosystems. We investigated the effects of mild hypoxia on the toxicity of copper to zebrafish (*Danio rerio)* embryos and larvae. Our data has shown that copper toxicity in embryos exposed from 4-28 hours post fertilisation (hpf) is significantly decreased under hypoxia. In contrast, for hatched larvae copper toxicity appears to increase when exposures occur under hypoxia. Under normoxia, but not under hypoxia, copper induced a delay in hatching when exposure occurred between 52 and 76hpf. Together, these data suggests that hypoxia influences the toxicity of copper to zebrafish embryos in a stage specific manner. Similarly to previous research, we have also observed that different stages of embryonic development had different susceptibility to copper irrespective of the concentration of oxygen in the water, with the first 24 hours and after hatching being particularly susceptible. We are now investigating the molecular mechanisms responsible for the alterations in copper toxicity observed under hypoxia.

TH017

Combined effects of copper and UVB radiation in single and multi-species algal assays

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In the impacted environment different variables play a role in determining the effects of pollutants on phytoplankton communities. Contaminants and physical-chemical factors might act as multiple stressors that simultaneously influence algal cells whereas algal species, besides having different sensitivities to the stressors, might positively or negatively interact e.g. competing for nutrients or releasing exudates in the surrounding medium. However, due to the difficulty of distinguishing between pollutant effects to single specie within complex communities, multi-species algal toxicity tests are rarely performed. In this study the single and combined effect of copper and UV radiation on monoalgal and mixed cultures of two microalgae was investigated. *Chlamydomonas reinhardtii* and *Synechocystis* sp. were exposed to $5x10^{-7}$M of $CuSO_4$, (representing respectively the EC10 and EC50) and to a light irradiation with strong UVB component (124 Wm^{-2} PAR, 5 Wm^{-2} UVA, 0.9 Wm^{-2} UVB). Effects of the two stressors alone and of their combination were evaluated on single and multi-species algal tests. Cellular traits (size, granularity, chlorophyll fluorescence), and oxidative stress were studied by flow cytometry. Growth inhibition and changes in the abundance of the two algal populations were also monitored. The results obtained for cells in monoalgal cultures were significantly different from the results obtained for the same specie exposed to the stressors in the mixed culture. Copper and UVR caused stronger effects in terms of growth inhibition, decrease in chlorophyll fluorescence and oxidative stress to *C. reinhardtii* cells in mixed cultures. Thus the presence of *Synechocystis* sp. seemed to render *C. reinhardtii* more vulnerable to the tested stressors. On the other hand *Synechocystis* sp. seemed to benefit by the presence of *C. reinhardtii,* with copper and UVR causing lower growth inhibition and lower oxidative stress. Combined exposure to Cu and UVR had synergistic effect on cells in both monoalgal and mixed cultures suggesting that the two stressors might interact in the same way independently of the complexity of the algal community. These results highlight the importance of taking into account algal-algal and multiple stressors interactions as variables to predict the cumulative effects of multiple pollutants on phytoplankton communities.

TH018

Influence of temperature increase on the response of river phototrophic biofilms to a chronic exposure to copper

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Agricultural rivers are highly affected by metal pollution, with copper being frequently detected. However, the pollution of surface waters must be viewed in a context of global change, and aquatic systems are generally subjected to multi-stress conditions, due to multiple chemical and/or physical pressures. Among the various environmental factors, a special attention should be paid to climatic changes, which can lead to rising temperatures. In lotic ecosystems, periphytic biofilms assume key ecological functions such as primary production and nutrient cycling. Indeed, even if effects of metals on microbial communities are relatively well known, there is a lack of data about the possible interactions between temperature increase and metal pollution. Accordingly, the present study aimed to evaluate in microcosms the influence of temperature increase on the response of biofilms to copper exposure (about 20 $\mu g\ L^{-1}$). Winter communities, collected in a 8°C water, were subjected for six weeks to four thermal conditions in presence or not of copper. At the initial river temperature (8°C), our results confirmed the chronic impact of copper on biofilms, both in terms of structure (biomass, distribution of algal groups, diatomic composition) and function (photosynthetic potential) throughout the study. At higher temperatures (13, 18 and 23°C), the effects were transient and varied according to the measured parameters. Statistical analysis revealed a significant interaction between temperature and copper. Furthermore, our study seemed to suggest that temperature can modify Cu bioavaibility. It is necessary to study these interactions in order to better understand theecotoxicological impact of metals in multi-stressed environments.

TH019

Natural variability of enzymatic biomarkers in riverine macrozoobenthos

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Biomarkers have been widely implied in ecotoxicology as indicators of exposure to several toxicants. Their use, coupled with observations at higher level of biological organisation, has the potential establish a mechanistic link between exposure to chemical stressors and consequent effects at population and community level. However, very often biomarkers are used by comparing different sites and looking for statistical significant differences among them, without taking into account the natural variability which may occur. Therefore, one of the major problems for the use of biomarkers in ecotoxicology is understanding if the measured responses are determined by the effects of stress factors or lie within the natural variability range produced by the effect of environmental parameters. This work aimed at evaluating the variability of some enzymatic biomarkers measured in freshwater benthic invertebrates collected, during 2012-2013, inpristine alpine streams in order to limit any potential anthropic influence. The experimental design considered: sampling sites in three different rivers; eight sampling dates covering the whole seasonal cycle; four taxonomic groups; four enzymes (Acetylcholinesterase, Glutathione-S-transferase, Alkaline phosphatase, and Catalase). Biomarkers levels were related to several environmental parameters (temperature, pH, conductivity, oxygen level, nitrogen and phosphate concentrations), to verify any kind of dependence. Data were elaborated using multivariate statistical methods. No evidence of human alteration were recorded, confirming the pristine status of the selected areas. Natural variability of enzymatic levels was found to be relevant across both space and time. Nevertheless, this variability was poorly explained by the monitored environmental parameters, and thus it is hardly predictable. The results of this work proved that great care should be paid when interpreting experiments in which biomarkers levels are measured and compared among sites or dates; further research is needed to understand how the natural variability of biomarkers could be accounted for and managed in ecotoxicological studies.

TH020
Invertebrate community response to water and sediment chemical composition in Mediterranean rivers
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River water is used for agricultural, industrial and domestic purposes that lead to water contamination with numerous natural and synthetic compounds. Emerging pollutants are a large and previously unknown group of compounds that are not totally removed by wastewater treatment plants (WWTPs) and can be found ubiquitously in natural waters. Although most of these compounds are present at low concentrations, many of them raise considerable ecotoxicological concerns, particularly when present as components of complex mixture (Loos *et al*., 2009). However, there is little information on their effects in freshwater communities. The objective of this study was to check the relationships between invertebrate communities and the presence of different groups of emerging pollutants in the water and in the sediment of 4 Iberian basins (Llobregat, Júcar, Guadalquivir, and Ebro). Four to six sites were sampled in each river during two consecutive years (2010, 2011) in early autumn. Five sediment samples were collected randomly with a core to obtain structural (species richness, diversity, density) and functioning parameters (catalasa activity) in invertebrate community. More than 800 pollutants, including pharmaceuticals, pesticides, illicit drugs, perfluorinated compounds and endocrine disrupting compounds (EDCs) were measured in water and sediment matrix. Physicochemical parameters were also measured in each site. Different statistical tools (Spearman's rank correlation, Redundancy Analysis and Generalized linear models) were performed in order to detect indicators of stress and risk on community. Different chemical composition was detected between sediment and water. Negative significant relationships were found between the abundance of invertebrate taxa and chemical concentrations. In water, pharmaceuticals and pesticides were the two families of pollutants which were repeatedly correlated with different genera. EDCs was also a group of compounds that determine invertebrate distribution in the most polluted sites. **References** Loos, R., Gawlik, B.M., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G.. *EU-wide survey of polar organic persistent pollutants in European river waters*. Environ. Poll. (2009), 157, 561-568.

TH021
EVALUATION OF PESTICIDE CONTAMINATION IN THE LLOBREGAT RIVER
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In this study a number of currently used pesticides were monitored and screened in Llobregat river ecosystem for two consecutive years 2010 and 2011. The levels of pesticides in water, sediment and biota were compared on two sampling periods. In water samples, the most frequent pesticides were imazalil, chlorpyriphos and diazinon that were in 93 % of the samples during the 2010 campain. The highest concentration was for malathion 320 ng L^{-1}. Nevertheless in 2011 campain, chlorpyriphos (80 %) or terbuthylazine-2-hydroxy (70 %) were the most ubiquitous and the maximum concentrations were detected for cabendazime (up to 697 ng L^{-1}) and diuron (up to 160 ng L^{-1}).

TH022
Low richness, high value: why and how to assess the ecological status of naturally saline streams of the western Mediterranean?
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Streams displaying high concentrations of dissolved salts (> 5,000 μS cm^{-1}) are quite common in the Mediterranean basin, as a result of water draining highly soluble lithologies in the arid catchments. These naturally stressed ecosystems are characterized by depauperate communities composed of habitat specialist species with a high degree of endemism and phylogenetic diversity. Most of these habitats are now endangered by the intensification of agricultural and urban uses within river catchments. Unfortunately, biomonitoring and conservation programs are ignoring these spots, which may lead to further loss of important proportions of exclusive biodiversity and ecosystem services. Therefore, it is crucial to develop an appropriate classification of these habitats and a bioassessment tool, which considers the ecological peculiarities of such ecosystems. Here, we attempt to address this challenge using a database of macroinvertebrate families and species collected in unpolluted streams covering the natural variability of western Mediterranean region (Iberian Peninsula, Morocco and Sicily). First, we performed a biological classification of the Iberian streams, which were also distinguished by their environmental features, obtaining seven types of freshwater (siliceous high-mountain headwaters, calcareous headwaters, calcareous mid-mountain and temporary) and saline streams (hyposaline, mesosaline and hypersaline). Second, we provided a method to assign such types to streams from the other studied regions according to key environmental variables (water electrical conductivity, basin area, site altitude, site mean annual temperature, basin lithology and reach hydrologic regime) by using regression trees. Third, we checked that stream types showed significant differences in community composition among and within regions at family and species level. Fourth, we tested the response of a wide range of metrics, including both traditional freshwater and novel saline-suited metrics, along gradients of land-use to select the best non-correlated indicators of alteration and propose a multimetric index to assess the ecological status of each saline stream type.

TH023
Effects of acute salinity exposure on invertebrate predation efficiency
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Salinisation is a growing global threat, recognized as one of the main stressors to freshwater ecosystems. Yet, its effects on the trophic structure of rivers and streams are poorly understood. We conducted a mesocosm experiment to test if elevated conductivities can induce a stress response in the invertebrate predator Erpobdellidae reducing its predation efficiency. The experiment was conducted at a flow-through mesocosm of 12 artificial stream channels relying on water pumped from a diversion channel of the Llobregat River (Barcelona, Spain). The pump provided a continuous supply of water to a 4000-L tank. A second 2000-L tank was filled with a salt-saturated solution (250 g l^{-1}), which was a mix of freshwater coming from the river and common table salt (NaCl). Taps on the inlet pipes regulated the proportions of river water and salt-saturated solution entering each of the mixing tanks, which created uniform conductivities in each tank before flowing into three artificial stream channels. We used two treatments levels: salt (nominal conductivity = 6 mS cm^{-1}) vs. control (nominal conductivity = 0.6 mS cm^{-1}), and Erpobdellidae presence vs. Erpobdellidae absence. Biomarkers were used to test the stress response of Erpobdellidae to the salt treatment. Invertebrates were collected from the artificial streams and chlorophyll *a* biomass was estimated. Preliminary results showed that salinity reduced significantly (P< 0.05, Student t test) antioxidant defenses, catalase activity, increased phase II detoxification metabolism, glutathione S transferase activity and induced oxidative stress measured as lipid peroxidation. These results thus indicate that the tested salinity levels could have detrimental effects on Erpobdellidae predation efficiency.

Acknowledgments Miguel Cañedo-Argüelles was supported by a Cátedra Principe de Asturias grant E-28-2012-0808040 (Fundación Española para la Ciencia y la Tecnología, Spain). Melissa Faria acknowledges the FCT fellowship SFRH/BPD/78342/2011, Portugal.

TH024
Sensitivity of microalgae to increased salinity under competition scenarios
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Algae, as producers, play a vital role in ecosystems by sequestering CO_2, through photosynthesis, and providing food and energy for consumers. Thus, they frequently constitute key components of water quality monitoring programs. Worldwide, low-lying coastal ecosystems are already facing salinization problems due to seawater intrusion. Salt stress can influence community composition by disrupting competitive relationships by, for example, favoring one species that under normal conditions is in competitive disadvantage. Also, it is known that some organisms may acquire tolerance when exposed to low levels of a chemical, which may influence their future permanence in an environment with salt intrusion. According, this work aimed at understanding the competitive outcome of two microalgae species under a scenario of exposure to low levels of salinity, without and with salt acclimation. For this purpose, the green algae *Pseudokerchneriella subcapitata* and *Chlorella vulgaris* were exposed, solely and jointly, to a control and to the EC_{25} of each species for growth inhibition to: 1) characterize the growth rate of both algae (when exposed solely and under competition) under continuous salt exposure (96h), 2) assess the growth rate, for the two scenarios mentioned above, after acclimation, for three generations, to low levels of salt. . Preliminary results showed that *P. subcapitata* exhibit a better performance comparatively to *C. vulgaris* when exposed under the control competition scenario. Conversely, *C. vulgaris* growth rate was always higher under scenarios of competition in the presence of salt stress. Similar results were obtained after acclimation of algae to low levels of salt. These results underline the susceptibility of the equilibrium of ecosystems, and, suggest that the contact with the toxic stress, for long periods of time, may cause shifts in the algae community structure. Further investigations must be performed to understand the dynamics of competition among producers, since they represent an important piece of the trophic chain.

TH025
Sublethal toxicity of chlorpyrifos to salmonid olfaction after hypersaline acclimation
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Acclimation to hypersaline environments enhanced the acute toxicity of certain organophosphate pesticides to euryhaline fish species; however sublethal effects have been far less studied. The present study focuses on the sublethal toxicity of chlorpyrifos to salmonid olfaction after hypersaline acclimation with the goal of linking molecular, physiological, and behavioural alterations. To determine molecular effects of combined exposure, coho salmon were acclimated to three different salinities (8ppth, 16ppth, 32ppth) for one week and mRNA was collected from the olfactory rosettes. Micrroarray hybridization was used to determine differences in gene expression for the different salinity treatments. Potential target genes involved in signal transduction, which have been shown to be impacted by chlorpyrifos in zebrafish, were identified and evaluated in coho salmon after saltwater acclimation. These genes included chloride interacellular channel 4, guanylate cyclase activator 1A (retina), guanine nucleotide binding protein (zgc:101761), calcium/calmodulin-dependent protein kinase II delta, and adrenergic alpha 2C receptor. To assess physiological effects, electroolfactograms (EOGs) were conducted on juvenile rainbow trout acclimated to freshwater and hypersalinity (16ppth) with co-exposure to environmentally relevant concentrations of chlorpyrifos (0.5 μg/L and 5.0 μg/L). Y-maze trough studies were used to assess preference and avoidance behavior of fish exposed to the same conditions as previously mentioned for EOG studies. Gene expression of the five target genes was examined in olfactory tissue from rainbow trout used in the EOG study. Exposure to hypersalinity and chlorpyrifos up-regulated all five target genes. Exposure to chlorpyrifos following acclimation to hypersalinity significantly decreased the response to L-serine and decreased the response to taurocholic acid additively. Avoidance of L-serine was decreased after chlorpyrifos exposure, as well as after concurrent exposure to both chlorpyrifos and hypersalinity. The combined results show that sublethal exposure to chlorpyrifos after hypersaline acclimation impacts salmonid olfaction, which may result from diminished signal transduction (NIEHS P30ES07033 and T32 ES018827).

Ecosystem structures and functions and their valuation in Ecological Risk Assessment (P)

TH026
Use of ecosystem services potentially affected by chemicals, for setting protection goals and the needs of risk assessment
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Assessing the risks of chemicals to man and the environment is based on the concept of comparing exposure to chemicals with their respective hazardous properties. However, there are differences in the criteria for deciding whether the level of exposure represents an acceptable or unacceptable risk. For man, decision criteria are focused on protecting the individual and regulations are applied relatively consistently around the globe. For the environment, protection goals are less clearly defined and not applied consistently in regional regulations. Regional environmental policies seem to take a cost-benefit approach to environmental impacts. There are two possible extremes for doing this: i) a precautionary approach aiming for zero release of chemicals into the environment; ii) uncontrolled release with no effective management to mitigate impacts. Most environmental regulatory schemes adopt an approach somewhere between these extremes. For example, some effects on individuals may be accepted if the population is unaffected or if it recovers from episodic exposure. For this approach to make sense, protection goals need to be suitably defined. Reviews of current regulations indicate that protection goals are only generally defined leaving a lack of clarity on how to achieve such protection. Discussion of current chemical regulation schemes has led to calls for changes in the way environmental toxicity thresholds are derived. In addition to this, there are policy discussions on going on the use of ecosystem services in setting protection goals. Examples include the Millennium Ecosystem Assessment, the European Commission strategy on biodiversity and ecosystem services, the UK National Ecosystem Assessment (UK NEA), the EFSA Guidance on a harmonised framework for pesticide risk assessment and the identification and evaluation of pesticide risk management options. This growing interest in using ecosystem services to help define and communicate protection goals will inevitably influence chemical regulation. Therefore, an ECETOC Task Force has been established to develop a structured framework for identifying which ecosystem services might be affected by chemicals, setting protection goals and guiding the focus of risk assessment.

TH027
Exploring the uncertainty of valuation techniques for the freshwater ecosystem services in the Llobregat river basin (NE Spain)
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Ecosystem services are the benefits that people receive from ecosystems. In order to calculate the total monetary value of a given ecosystem, each benefit should be valued and sumed up as to give a Total Economic Value (TEV) of the valuated ecosystem. To date, benefit-values have been largely expressed in biophysical units (e.g. m^3 for water supply) which, due to a lack of homogeneity in the units, cannot be compared and summed up in as TEV. Expressing the benefit-values of an ecosystem in monetary terms appears to be useful as this metric is meaningful to stakeholders and gives unequivocal support to the belief that ecosystems have a significant, positive social contribution. However, several methods can be used to value a given benefit, and several authors have already shown large discrepancies in the ecosystem values, depending on the used method (e.g. Bockstael et al., 1989). The aim of this study is (1) to explore the uncertainity in the valuation of ecosystem services, given the multiple benefits one might consider for a given service, as well as the multiple metrics that can be used for a given benefit, and (2) to explore the transferability of the benefit-values estimated in one study area, the Llobregat river basin, to the valuation of other basins' ecosystems, given the uncertainty of each benefit-values. The local values of biophysical parameters are obtained from the application of the Integrated Valuation of Ecosystem Services and Tradeoffs model (InVEST) for the assessment of 4 freshwater ecosystem services (water provisioning, erosion control, water purification, and habitat for species) provided across the Llobregat basin. The local monetary values are obtained based on the local Net Present Values of the services or are gathered from an extensive literature review through benefit-transfer approach. The Economic Value (EV) of each

ecosystem service ($EV_{water\ provisioning}$, $EV_{erosion\ control}$, $EV_{water\ purification}$ and $EV_{habitat\ for\ species}$) are aggregated into a single TEV among the Llobregat basin. This single TEV is actually partial TEV as we are just considering 4 freshwater ecosystem services under this study. *Acknowledgement* - The authors thank the Spanish Ministry of Economy and Competitiveness for its financial support through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065).

TH028

National screening on selected organic compounds in groundwater

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In 2011, the French Ministry of Ecology and the Water and Aquatic Environment National Office have implemented a national reconnaissance effort targeting "emerging" organic contaminants with various potential uses and origins. This study presents this national screening and fully discusses the occurrence of selected organic compounds (OCs) in groundwater. 411 OCs, including parents and transformation products (TPs), were targeted because they were not or poorly monitored in French groundwater. Ninety-one of the selected OCs are referenced as hazard molecules (WFD). They were collected in groundwater on 494 sites (springs, wells and boreholes) across France during two national sampling campaigns in spring and fall 2011. Amongst all sites, 282 are directly used (untreated) to provide drinking water. Groundwater samples were analysed in two French routine laboratories that are currently in charge of the regulatory monitoring. Amongst the 411 monitored substances, 180 were detected in at least one sampling point with values greater than the minimum reporting level, representing 44% of monitored compounds. Sixty substances were pharmaceutical products, 63 industrials products, 43 pesticides and 14 other emerging contaminants. Fifty-five compounds are found in more than 1% of collected samples. Although these products, industrial compounds constitute the most detected group with 23 compounds detected compared to 13, 14 and 5 pharmaceuticals products, pesticides and others emerging contaminants respectively. The 3 most frequently detected compounds are 1,2,3,4,6,7,8-H7CDD (61%, dioxin), deisopropyl-desethyl-atrazine (41.4%, forbidden herbicide metabolite) and caffeine (39.8%, life style product). The risk assessment associated with the occurrence of these substances will be fully discussed. Ultimately, these results should help the water resource managers and environmental regulators to develop sound policies regarding the occurrence and distribution of "emerging" organic contaminants in groundwater.

TH029

Selection of representative arthropod species for risk assessment and field studies for Plant Protection Products

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The present risk assessment scheme is based on a simple calculation of a hazard quotient HQ (or TER in Germany) for a handful of arthropod species. While the standard species used in risk assessment (only *Aphidius rhopalosiphi* and *Typhlodromus pyri* at Tier 1; plus at Tier 2, two from a choice of *Chrysoperla carnea*, *Poecilus cupreus, Aleochara bilineata, Episyrphus balteatus, Coccinella septempunctata*) were selected at least partly because they can easily be cultured in the laboratory, in some higher tier studies (including refined exposure scenarios, modelling or field studies) with a closer focus on delivery of ecosystem services, it is often debated which arthropod species or group should be considered as relevant. The main concern is often that it is difficult to choose one representative species from the huge number of arthropod species available, with very different life cycles, reproduction, longevity and behaviour. An approach used by some regulatory authorities is to combine worst-case features into a hypothetical 'vulnerable' species, e.g. taking low survival from one species, low reproduction from another species, etc. This usually results in a hypothetical worst-case species, which could not survive in reality. We therefore propose a new, more realistic approach to select representative species. This approach is based on analysis of key species traits, such as reproduction, dispersal, morphology and abundance and aims at identifying a species that "covers" 90% (or any other given fraction) of all species with regard to their susceptibility. We exemplarily show how this generic approach could be applied in field studies or refined risk assessments.

TH030

Biogeochemical changes, microbial activity, and ecosystem services, in eutrophic wetlands with and without vegetation: a mesocosms experiment focused in the nitrogen cycle

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Wetlands are among the most affected ecosystems by eutrophication, and this arise environmental risks due that these environments have important ecological services. However, wetlands are also systems with high capacity to reduce the content of harmful substances in the water, and play a key role against pollution. Changes in the biogeochemical conditions in the water-soil-plant system influence the response of these systems, and hence in their capacity to preserve the ecological services and environmental quality. In this work we studied the behaviour of wetlands against nitrogen-enriched water, with and without plants, during alternating flooding and drying phases in experimental mesocosms. The mesocosms (1x0.5x0.5 m) were filled with sand at the bottom, and soil above the sand, to simulate the two typical soil layers in many coastal wetlands. These mesocosms were equipped with Rhizon type samplers at the two depths (to extract pore water) and Eh/pH electrodes. We assayed treatments with common reed (Phragmites australis) and without plants. The systems were flooded for three-four weeks and then drained to dry them. We used two types of eutrophic water. Water with 20 mg/L of nitrates and water with 200 mg/L of nitrates. The cycle was repeated three times during 29 weeks. We monitored pH and Eh and measured the content of nitrates and ammonium in the pore water and lixiviates, and N2O emissions. Also, we measured microbial biomass and enzimatic activity in the soils. The results indicated that Eh and pH were strongly influenced by the flooding-drying conditions, with strong differences in the response of the surface and subsurface layers, and that Phragmites and the type of water modulated this behaviour. The capacity of the experimental wetlands to depurate the water was correlated with changes in the biogeochemical conditions, the presence-absence of plants and the period of the year. The microbial activity was also influenced by the different treatments, with a strong decrease attributable to the death of the micro-organisms during the strongest anoxic phases.

TH031

Occurrence and removal of UV filters in urban groundwater of Barcelona (Spain)

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The occurrence of emerging pollutants in wastewater and sludge from WWTPs has caused much attention in recent years. Since current treatment techniques do not efficiently remove these chemicals [1,2], they enter the environment, even reaching groundwater [3]. So far, most of the published reports are focused on the behavior of the emerging pollutants in WWTPs, but little is known about alternative strategies for their removal. In this study on one hand we report for the first time the occurrence of UV filters (UV F), in urban groundwater. On the other hand, we point out the effectiveness of the redox processes taking place to degrade/remove the UV F entering the aquifers. Specifically, the presented work is concerned with the occurrence of UV filters in urban aquifers in connection with (1) the spatial distribution of UV F in Barcelona's groundwater, (2) the depth of the groundwater, (3) the physicochemical properties of the target compounds, (4) the recharge sources, and (5) the redox conditions of the aquifers. The highest groundwater concentrations and the largest number of detected UV F were observed in an aquifer recharged by a polluted river (ca 55 ng/L). In contrast, urbanized areas presented lower concentrations. Two pathways can be identified for UV F to enter the aquifers: leakage from sewage network which contains WWTP influents in urbanized areas and WWTP effluents discharged into a river. Concentrations of UV F estimated mainly from the wastewater fraction in groundwater samples were significantly much higher than the measured ones, suggesting that UV F might undergo transformation processes in both reducing and oxidizing conditions. According to our results, aquifer natural attenuation might be considered as a promising alternative to remove UV filters, and likely other organic emerging pollutants from water. **References** [1] W. Li, Y. Ma, C. Guo, W. Hu, K. Liu, Y. Wang, T. Zhu, Water Res. 41 (2007) 3506. [2] P. Gago-Ferrero, N. Mastroianni, M.S. Díaz-Cruz, D. Barceló, J. Chromatogr. A 1294 (2013) 106. [3] A. Jurado, N. Mastroianni, E. Vàzquez-Suñé, J. Carrera, I. Tubau, E. Pujades, C. Postigo, M. López de Alda, D. Barceló, Sci. Total Environ. 424 (2012) 280.
Acknowledgements: The Spanish Ministry of Economy and Competitiveness for the projects MEPONE (BIA2010-20244) and SCARCE (Consolider-Ingenio 2010 CSD2009-00065), and the Generalitat de Catalunya (2009-SGR-1057 and 2009-SGR-965).

TH032

An integrated historical & ecological perspective to interpret the evolution of water quality in European metropolitan rivers.

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In the last 15 decades, the enormous growth of the metropolitan aggregations

developed along fluvial axis caused a high impact on rivers. An interdisciplinary group has collected chemical, biological, ecological, geographical and historical information to reconstruct the responses of rivers to changes in environmental pressures in four European study cases: Seine (Paris), Seine (Brussels), Spree (Berlin) and Lambro (Milan). The integration of historical data on monitoring and management institutions, together with the analysis of water quality information, provided evidence for the evolution of environmental awareness in the first half of XX century. On the contrary, in the second half, a common trend of water quality amelioration status has been detected in all case studies, and was related to the general advancement of ecological competencies. Specific patterns of deviation from this common trend were highlighted in each cases, and discussed under the integrated approach, delineating the main drivers at the base of quality evolution. Focusing on the Lambro river study case, synthetic descriptors of biological quality were estimated by harmonizing recent and historical data. The main factors at the base of fish and macroinvertebrates communities shift, partially depending on the evolution of chemical status, but also on factors acting on upper basin scale, were individuated.

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (P)

TH033

The surface-charge dependent cellular apoptosis induced by engineered silver nanoparticles

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Silver nanoparticles (AgNPs) are being used widely in medicine and consumer products. There is an increasing concern about the potential risks of AgNPs to the human health and environment after its eventual release into the environment. Many studies had showed that the toxicity of AgNPs was relevant to many factors such as particle size, surface coating and shape. However, an important factor-surface charge-leading to the toxic mechanisms of AgNPs is unknown. In the present study, the uptake and apoptosis mechanisms of AgNPs with different surface charge to mouse hepatoma cells (Hepa1c1c7) were studied. They were negative surface charge of citrate coated AgNPs (Citrate AgNPs), polyvinylpyrolidone coated AgNPs (PVP AgNPs), polyethylene glycol (PEG AgNPs) and positive surface charge of branched polyethyleneimine coated AgNPs (BPEI AgNPs). The cell viability was measured by ATP assay which was observed luminescence of proportional to the quantity of ATP in cells. The uptake of AgNPs was determined with transmission electron microscopy (TEM) and inductively coupled plasma mass spectrometry (ICP-MS). Cellular apoptosis, in terms of mitochondrial membrane potential ($?\Psi m$), DNA fragmentation, as well asBcl2 and Caspase-3 gene expression was tested after exposure 5μg/ml AgNPs 24h. The early results demonstrated that the positive surface charge AgNPs showed significant higher toxicity to Hepa1c1c7 compared to the negative surface charge AgNPs even the uptake amount of the AgNPs into cells were similar.

TH034

Comparative study investigating the differences in sensitivities of an array of rainbow trout cell lines to primary hepatocyte cultures following AgNP insult.

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Among all the classes of nanomaterial's AgNPs have potentially an important ecotoxicological impact due to the hazard associated with these particles reaching freshwater environments. Fish are particularly susceptible to the toxic effects of metal ions such as silver and with knowledge gaps surrounding the contribution of dissolution and unique particle effects they represent a group of a vulnerable organisms. Using the rainbow trout (*Oncorhynchus mykiss*) as a test species we have employed both primary cultures and cell lines to test the cytotoxicity of AgNPs and $Ag(NO_3)_2$. Both primary cultures and cell lines represent valuable in vitro models to facilitate hazard ranking and to prioritise for in vivo testing. However there is a debate surrounding the suitability and sensitivity of these models for toxicity testing. Some of the drawbacks surround the loss in biotransformation activity and cell to cell contact which can influence how cells would respond to toxic compounds on an organism level. Therefore in this study we have used an array of cell lines (RTL-W1 hepatic cells, RTH-149 hepatoma, RTG-2 gonad) and compared sensitivities to primary rainbow trout hepatocyte cultures. We have employed a 3 in 1 fluorometric based assay system thatincorporates a simple and robust 96-well plate format and provides a means to characterise cytotoxicity in terms of metabolic activity, cell membrane integrity and lysosomal damage. Interestingly the RTH-149 hepatoma cell line proved to be the most sensitive towards the toxic effects of AgNPs, with an IC50 of 19.8 μg/mL. The primary hepatocytes IC50 was 30.6 μg/mL. The IC50 for the RTL-W1 and RTG-2 cell lines were 75.9 and 41.7μg/mL respectively. As well as allowing us to determine differences in sensitivities among cell lines we were able to determine if the AgNPs were invoking different cellular responses depending on the model used. For example the RTL-W1 cell line was more susceptible to lysosomal damage than to alterations in metabolic activity following AgNP insult.Such a comparative study provides information on the appropriateness of specific in vitro models as test systems. Information obtained can be used to interpret potential whole organism toxicity, for nanoparticle toxicity screening and hazard assessment.

TH035

Silver Nanocolloids Have Impacts on Medaka Innate Immune Responses

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Silver nanomaterials have been become one of the major components of international healthcare markets. Their environmental fate and ecological risks are not understood yet. Impacts of silver nanomaterials on environmental health sciences has been internationally concerned and discussed in the OECD. Regarding toxic effects of silver nanomaterials in fish, lethal toxicities, inhibited embryo developments, uptake of silver, injuries of liver and gills, and induced oxidative stress, MTs and HSPs have been reported. However, there is little information of specific toxic mechanism of silver nanomaterials. In our previous studies, medaka larvae were exposed to 0.05 mg/L of silver nanocolloids (SNCs) and then subjected to microarray analysis (total 5901 genes). 102 genes relative to signal transduction, protein syntheses, transcription, and immune response were revealed to be suppressed and shared 72% of total suppressed genes. On the other hands, 51 genes relative to the same above categories were enhanced and shared 53% of total enhanced genes. Genes for immune response were included as 14% of all suppressed genes, and 16% of all enhanced genes. This microarray data suggested that immune is probable target of SNCs toxicity. In order to investigate exposure effects of SNCs on medaka immune system, medaka embryos were exposed 0.5 mg/L of SNCs until hatching; and then, gene expression levels of nuclear factor kappa B (NFκB), which is transcription factor that induce proinflammatory cytokine, and tumor necrosis factor α (TNFα) were measured by qRT-PCR. Both gene expressions were increased by SNCs exposure. These factors are well known to be regulated by toll-like receptors (TLRs) signaling in innate immune. This study will introduce toxic effects of SNCs on innate immune system using medaka embryo and larvae.

TH036

Toxicity screening of silver nanoparticles to Eisenia fetida earthworm through standardized OECD test and in vitro assay with coelomocytes

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In recent years, the use of silver nanoparticle (Ag-NP) containing products has greatly increased, so the release of Ag-NPs into different environmental compartments is already occurring. Thus, the concern about the still scarcely known hazards of nanosized materials to environmental health is growing. NP potential risk has been mainly studied in aquatic environments, while their effects in soils are poorly investigated despite the great complexity of soil matrix and the potential interactions of soil components with NPs that could affect their toxicity. *Eisenia fetida* earthworm is a model terrestrial organism that has been broadly used due to its sensitivity to different toxicants in standardized toxicity tests. Among them, the Filter Paper Contact Toxicity Test (OECD-207) is an initial screening method to identify toxic substances and to obtain relevant toxicity data (LC50,EC50). Recently, *in vitro* tests have also been developed as rapid and cost-effective methods for toxicity assessment of chemicals. Therefore, the aim of this work is to test the toxicity of Ag-NPs to *E. fetida* through the Filter Paper Contact Toxicity Test (*in vivo*), complemented with *in vitro* tests of coelomocytes. For *in vivo* testing, earthworms were exposed on moist filter paper to PVP-PEI coated Ag-NPs and to PVP-PEI agent separately in 0-0.2mg/cm^2 range concentration. After 48h mortality and weight loss were assessed, together with Ag accumulation measurements in earthworm tissues. In addition, number and size of mucocytes found in the epidermis were addressed after Alcian Blue staining. Regarding *in vitro* screening, coelomocytes were exposed to 0-1mg/l PVP-PEI Ag-NP and 0.0001-10,4mg/l PVP-PEI and Neutral Red Uptake assay was performed after 24h. Coated Ag-NPs caused 100% mortality at 0.2 and 0.02mg/cm^2 and a dose-dependent weight loss (up to 43.54%) likely due to a dehydration effect caused by nanoparticles which included also an increase in mucocyte secretion. PVP-PEI agent appeared to be less toxic than coated Ag-NP since total mortality was only found in 0.2mg/cm^2 and a severe weight loss was only recorded in

0.02mg/cm^2 treatment (47.51%). *In vitro* screening evidenced PVP-PEI not to be cytotoxic while coated Ag-NPs exposure exerted an initial stress at low doses and severe toxicity at highest concentrations. The obtained results prove the usefulness of *in vivo* and *in vitro* toxicity test in *E. fetida* for the assessment of the potential risk of Ag-NPs in soil ecosystems.

TH037

Effects on metallothionein transcription levels and histopathological alterations in mussel gills after in vivo exposure to metal nanoparticles (CuO, Ag, CdS).

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Keywords: Nanoparticles, mussel, gills, metallothioneins. In spite of the wide use of nanotechnology and nanomaterials and the profits achieved for daily life during the last decade, few is known about their toxicity and effects on the marine environment and biota. Mussels *Mytilus galloprovincialis* are suitable sentinel organisms to assess the toxicity and accumulation of contaminants, including nanoparticles. Mussel gills are a key organ involved in different processes related with the uptake and transport of contaminants. In the present study mussels *M. galloprovincialis* were exposed to different metal containing nanoparticles (CuO, CdS y Ag) and to their respective bulk and aqueous forms for 21 days in order to study metal accumulation and alterations at different levels of biological complexity, say, transcriptional levels of two metallothioneins (*mt10* and *mt20*), metal distribution in different target cell-types (autometallography) and histopathological alterations at tissue level. The exposure to different forms of Cd produced an early accumulation of metals in different gill cell types accompanied byincreased *mt20* levels, at 1 and 21 days of exposure, that was followed by several histopathological alterations at 21 days (reduction of epithelium thickness at endothelial zone and large hemocytic infiltration). On the other hand, CuO and Ag NPs, and their respective bulk and aqueous forms, produced a variety of histopathological alterations(ciliar erosion, reduction of epithelium (endothelium) thickness, increased mucocytic proliferation and hemocytic infiltration),although differences at *mt* transcription levels and accumulation of metals were not registered. These results suggest that NPs have different effects, but in general a cause-effect relationship between the changes observed at transcriptional and cellular level, with the histopathological alterations produced by NPs and their respective bulk and ionic forms can be envisaged. Acknowledgements: EU 7th FP (NanoReTox, ref CP-FP 214478-2), Spanish Ministry Sci & Technol (NanoCancer CTM2009-13477) & Economy and Competitivity (NanoSilverOmics MAT-2012-39372), Basque Gov. (Consolidated Res Grp (GIC07/26-IT-810-13), UPV/EHU: Unit of Formation and Research *"Protection of Ecosystem Health"* (UFI11/37).

TH038

Trophic Transfer of Silver Nanoparticles from Artemia Nauplii to Marine Medaka

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There has been increasing concern for the potential threats of silver nanoparticles (AgNPs) to aquatic organisms. Our previous study has shown limited uptake of citrate coated AgNPs from water in marine medaka (*Oryzias melastigma*).The present study investigated the trophic transfer of AgNPs from Artemia nauplii (*Artemia salina*) to medaka. We found that the dissolved uptake of AgNPs in Artemia was size-dependent. The aggregated AgNPs (20 nm and 80 nm) and well dispersed 80 nm AgNPs (stabilized by 20 µM Tween 20) were readily accumulated by Artemia after 24 h of waterborne exposure (BCF>5 L/g), while little well-dispersed 20 nm AgNPs was accumulated (BCF3-contaminated Artemia (11 µg/g) in the first week. Further study on the biotransformation and translocation of AgNPs in the digestion tract is needed to explain such difference.

TH039

Comparative toxicity of Nano- and Microscale Copper Oxide Particles in Marine Mussels

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Copper oxide nanoparticles (CuO NPs) are one type of NP used in a wide variety of applications, for example, batteries, semiconductors, electronic chips, inks and heat transfer nanofluids. The growing use of CuO NPs has given rise to worldwide concerns regarding their environmental release, particularly to the marine environment. The toxicity of CuO NPs on organisms and human health is poorly studied compared to other metal oxides such as ZnO or TiO2. Hence, it is essential to investigate CuO NP exposure, uptake and effects on key marine organisms, such as benthic filter feeders and compare their effects with those of CuO microparticles (MPs). Very few studies have determined the effect of CuO NPs on mussels, and these have concentrated solely on oxidative stress and lipid peroxidation, but have not investigated DNA damage or cell viability. As CuONPs are likely to agglomerate in seawter comparative toxicity data for copper oxide microparticles are lacking for copper oxide microparticles as well as their respective effects on filter-feeding marine mussels.\nTwo species of marine mussel (Mytilus edilus and Modiolus modiolus) were exposed to CuO NPs and CuO MPs at the following concentrations 5, 10, 15 and 20µgL-1 along with the comntrol; for 72 hours, endpoints included, DNA stand breaks (Comet assay), cell viability (flow cytometry) and oxidative stress (superoxide dismutase (SOD) activity). The blue mussel, M. edulis was selected due to its wide distribution and sensitivity. The horse mussel, M. modiolus, was selected as a comparison, because of its ecological role in establishing biogenic reefs, which are increasing being classified as special marine features and require protection.\nResults showed that both forms of CuO (NPs and MPs) can cause DNA damage in both types of cells (haemolymph and gill) for both mussel species in a concentration-dependent manner. Similarly, both forms of particulate CuO displayed the potential to decrease the cell viability in haemolymph cells for both mussel species. Both forms of CuO (NPs and MPs) have the potential to increase SOD activity, indication an induction of oxidative stress.\nM. edulis mussels are more sensitive to both CuO forms (especially MPs) than M. modiolus mussels. The significance of these effects is influenced by many factors such as particle size, the length of the exposure, the soluble Cu in the medium and the accumulation of CuO particules in the species. Finally, these initial findings indicate that filter-feeding mussels are potential targets for nanoparticle exposure and suitable biomarker organisms in the marine environment. Keywords: Copper oxide, nanoparticles, marine systems, marine mussels\n\nI acknowlege my repective funder for this project Jazan University, Jazan, Saudi Arabia.

TH040

Ecotoxicity of nanomaterials in relation to the freshwater blackworm Lumbriculus variegatus

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Rapid growth in the field of nanotechnology is ever increasing the potential release of nanomaterials (NM) into the environment. As the main recipient of industrial and domestic wastewaters, the fate and behaviour of NMs in aquatic systems has come under much scrutiny in recent years. Sediment habitats in particular are anticipated to be the final sink for NMs due to the processes of aggregation, agglomeration and sedimentation, however, little is still known in terms of the effects on sediment fauna. This study, funded by the FP7 MARINA project aims to investigate the toxicity of two reference engineered nanoparticles; silver (NM300K) and titanium dioxide (NM104) towards the freshwater, sediment ingesting oligocheate, *Lumbriculus variegatus*. Toxicity tests including *L. variegatus* survival, reproduction, biomass and behaviour were carried out in both formulated sediment (28 days) and medium (96 hours), following OECD 225 guidelines. Alterations to abiotic conditions (natural organic matter content and pH) proved to influence the toxicity of NM300K in both medium and sediment. Antioxidant enzyme assays (catalase and superoxide dismutase) were used to assess the toxic action of nanoparticles towards *L. variegatus,* whilst lipid peroxidation was determined as an indicator of cell damage. NM300K proved to be the more toxic reference NM in both sediment and medium tests (LC_{50} values of 1000 mg/kg dry sediment and 497 µg/L respectively). However,toxic effects induced by NM104 were only recorded at the top concentration of sediment exposures (2000 mg/kg dry sediment), whilst no effects were observed in medium exposures up to 1.5 g/L.

TH041

In vivo effects of zinc oxide nanoparticles to the marine bivalve Venerupis philippinarum

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Nowadays, nanotechnology is a major innovative scientific and economic growth area. Its rapid expansion has led to a large increase in the number of products containing nano-sized materials. The increased production, use and application of nanoparticles (NPs) in several consumer products raise concerns for environmental release and subsequent impacts in natural communities. However, little is known about the potential toxicity or fate of NPs in the aquatic biota. As ultimate sink for most contaminants, marine coastal environments are susceptible to NP exposure and filter-feeding invertebrates, bivalve molluscs in particular, represent a target for NP toxicity. Therefore, efforts should be addressed to the evaluation of the biological impact of NPs in these ecologically and economically relevant organisms. In this work, the in *vivo* effects of zinc oxide nanoparticles (nZnO), one of the most widespread NPs in use, were investigated in the bivalve, *Venerupis philippinarum*. Clams were exposed under semi-static conditions for 7 days to different sublethal concentrations of nZnO suspensions (0, 1, 10 ug/L) and $ZnCl_2$ (10 ug/L). $ZnCl_2$ was used to investigate possible prevalent contributions of Zn^{2+}

release to nZnO toxicity. At differing time intervals during the exposure, several cellular and biochemical responses were evaluated in clam haemocytes, gills and digestive gland. A significant decrease in gill AChE activity was observed in all the experimental conditions tested, suggesting the possible role of zinc ions in nZnO toxicity. Significant increases in catalase and superoxide dismutase activities, and a decreasing trend of glutathione S-transferase activity, indicated the involvement of oxidative stress in nZnO toxicity. Of the various immunomarkers measured, haemocyte proliferation increased significantly suggesting stress conditions in clams. DNA damage in haemocytes was also revealed by SCGE assay at both nZnO concentrations tested. Different responses in nZnO and $ZnCl_2$ exposed clams suggested different mechanisms of action the contaminants, nZnO toxicity possibly depending not only on release of zinc ions, but also on the particular characteristics of NPs (e.g., size, surface, shape).

TH042

Particles toxicity: just a matter of size?

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Nowadays engineered nanoparticles represent innovative goods in various fields, from electronics to manufacturing, from medicine to agriculture. Studies addressing the toxicity of nanomaterials have found that smaller sized nanoparticles are usually more toxic to organisms than those having a larger size. Aim of this study was to verify the hypothesis of a size-dependent toxicity, by comparing the toxicity to marine crustaceans of metal particles of micro and nano size. Larvae of *Artemia* sp. and nauplii of *Amphibalanus amphitrite* were exposed to titanium dioxide (TiO_2) and silver (Ag) particles of nano and micro size (n-TiO_2, m-TiO_2, n-Ag and $AgNO_3$) for 24 and 48 h in seawater, with suspensions from 0.01 mg mL^{-1} to 1 mg mL^{-1} for TiO_2 and 0.1 mg L^{-1} to 50 mg L^{-1} for Ag. After the exposures, acute and behavioural responses (mortality and swimming speed alteration) were evaluated as end-points. Results indicated that both end-points were able to underline toxic effects caused by n-Ag and $AgNO_3$; these effects were quantified by means of LC_{50} and EC_{50} values, when possible. Conversely, n-TiO_2 and m-TiO_2 did not cause any toxic effects on on *A. salina* and *A. amphitrite* independently from the size. Considering metal nanoparticles, the Ag toxicity seems to be not size dependent when considering the effect on *A. amphitrite* nauplii whereas the n-Ag shows a toxic effect on *A. salina* stronger than that of $AgNO_3$. On the basis of obtained results, we do not allow to reject or verify the hypothesis of a size dependent toxicity; in fact, results suggest that the size effect is compound dependent and, when existing, it is species dependent.

TH043

Identification and avoidance of potential artifacts in nanomaterial ecotoxicity measurements

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Novel physicochemistries of engineered nanomaterials (ENMs) offer considerable commercial potential for new products and processes, but also the possibility of unforeseen and negative consequences upon ENM release into the environment. Investigations of ENM ecotoxicity have revealed that the unique properties of ENMs and a lack of appropriate test methods can lead to results that are inaccurate or not reproducible. The occurrence of spurious results or misinterpretations of results from ENM toxicity tests (defined here as experimental artifacts) that are unique to investigations of ENMs (as opposed to traditional toxicants) have been reported, but have not yet been systemically reviewed. Our objective in this manuscript is to highlight artifacts that can occur at each step of ecotoxicity testing: procurement or synthesis of the ENMs and assessment of potential toxic impurities such as metals or endotoxins, ENM storage, dispersion of the ENMs in the test medium, direct interference with assay reagents and indirect effects such as nutrient depletion during the assay, and assessment of the ENM biodistribution in organisms. We recommend thorough characterization of initial ENMs including measurement of impurities, implementation of steps to minimize changes to the ENMs during storage, inclusion of a set of experimental controls (e.g., to assess impacts of nutrient depletion, ENM specific effects, impurities in ENM formulation, desorbed surface coatings, the dispersion process, and direct interference of ENM with toxicity assays), and use of orthogonal measurement methods when available to assess ENMs fate and distribution in organisms.

TH044

Contrasting hazard data across a range of species and endpoints – lessons for nanosafety

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A variety of nanoparticles are used as pigments in paints and dyes and for medical applications. Exposure to these particles can be experienced in an occupational setting during their production and incorporation into the finished product, application and refinement (such as sanding), as well as by the end user. Nanoparticulate pigments can also reach the environment through waste, spills, abrasion and wash-off. In this project we studied the biological effects of a range of nanoparticles used in the pigments and paints industries. In our work we used a range of species (from environmental to human models) and endpoints, from *in vitro* to *in vivo*, at both acute and chronic level. Results have allowed us to rank the hazard of the particles across those models and approaches and also to compare acute and chronic results, as well as in vitro and in vivo. Thorough characterisation of particles in water, as well as relevant exposure media, at different time points and concentrations, allowed relationships to be established between exposure and effects. The particles used include silver (Ag), titanium dioxide (TiO_2), aluminium oxide (Al_2O_3), cobalt aluminate spinels ($CoAl_2O_4$), cadmium selenide core quantum dots with a zinc sulphate shell (CdSe/ZnS), iron oxide (magnetite, Fe_3O_4) and zinc oxide (ZnO). The environmental test organisms are *Daphnia magna* (water flea), *Pseudokirchnerialla subcapitata* (microalga) and *Lumbriculus variegatus* (blackworm). We have also tested on standard human cell lines and on mice. The project aims to concentrate on the most hazardous particles and surface modify them to reduce adverse effects in the test models while preserving the properties which make them valuable as paints and dyes. The collaboration with the industrial partners, the combined information on human and environmental hazards, as well as the facilities to modify particles to keep them suitable for their intended application while minimising the hazard to environmental and public health make this project very valuable for investigating realistic steps towards the safe and sustainable use of nanomaterials.

TH045

Nanoparticle ecotoxicity testing in laboratory media and wastewater using a lux-based switch-off Pseudomonas putida bioreporter.

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Introduction: Widely used in industrial sectors and commercial products, inorganic nanoparticles pose growing concern regarding their potential release and impact to the environment. However, information on their fate, bioavailability and ecotoxicity, especially in real environmental matrices, is still limited. Bacteria have been successfully used, notably *Escherichia coli*, as bioreporters for drug and chemical testing in the past two decades, usually via the indirect measurement of emitted luminescence from genetically modified microorganisms. However, examples of nanoecotoxicological studies based on such bioreporters, especially other than *E. coli*, remain scarce. **Methods:** Toxicity effects of three standard inorganic nanoparticles (e.g. Ag NM-300k, ZnO NM-110, and TiO_2 NM-104 from OECD) have been assessed in laboratory medium, artificial and real wastewater via switch-off based planktonic assays with a luminescent *Pseudomonas putida* bioreporter originally isolated from a wastewater treatment plant. Nanoparticles have been characterised by Dynamic Light Scattering, UV-Visible and Atomic Absorption Spectroscopy carried out in matrices of exposure. **Results:** High, medium, and low toxicities characterised by specific IC_{50} values at 1 h of < 5 mg/L, ~100 mg/L, and > 200 mg/L were observed on exposures of *P. putida* bioreporter to respectively Ag NM-300k, ZnO NM-110, and TiO_2 NM-104 nanoparticles in laboratory medium. Similar results were obtained for Ag NM-300k and TiO_2 NM-104 in artificial wastewater whereas major differences were observed for ZnO NM-110 (IC_{50} at 1 h > 200 mg/L). Toxicity effects were then closely related with changes in nanoparticle physico-chemical properties, especially in terms of agglomeration states and dissolution rates, function of the matrix of exposure. Investigations with spiked real wastewater are currently underway. **Conclusions:** In this study we demonstrate the suitability of an eco-relevant *P. putida* bioreporter to the assessment of nanoparticle effects, the importance of characterisation information in matrices of exposure, and further highlight key challenges of working with real environmental matrices. **Acknowledgement:** Heriot Watt University for fundings (James Watt Studentship). **Keywords:** Inorganic nanoparticle, Ecotoxicity testing, *Pseudomonas putida*, Wastewater.

TH046

Toxicity of engineered nanomaterials in an environmental model, the nematode Caenorhabditis elegans

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Although nanotechnology has expanded substantially in recent years, there is still much lack of information on the safety of engineered nanomaterials, particularly given their wide use in consumer products which may lead to potential risks to the environment. In this study, the toxicity of selected engineered nanomaterials is assessed by focusing on the viability of the test organism, *C. elegans*. To define the concentrations that lead to 50% death of the test animal (LC_{50}) in a specific time exposure, four strains of *C. elegans* carrying the absence of particular genes were

exposed to different concentrations of test substances for 24 hours in the presence of bacterial food source (*Escherichia coli*). The LC_{50} values of nanoparticulate silver of different morphologies (rods and spheric) were compared to its dissolved ion counterpart on the four selected strains. Comparison of sensitivity across the different strains of *C. elegans* allows the establishment of the potential mechanistic pathway(s) of toxicity arising from exposures to these nanomaterials. Dissolved silver ion was found to be the most toxic form with LC_{50} values (24 h) of 166.75, 255.01, 162.44, and 236.99 μg/L for N2 (wild-type), MT1522 (loss of pro-apoptotic gene), VC433 (loss of gene encoding superoxide dismutase), and VC128 (loss of gene encoding metallothionein enzyme), respectively. In contrast, the nanoparticulate forms, silver rods (OECD NM302) and silver nanomaterials (OECD NM300K) showed the higher LC_{50} values in all four strains of *C. elegans*. However, the difference in LC_{50} values of test substances in each strain was not enough to draw a conclusion, at this stage, of potential mechanism(s) of toxicity. Therefore, further studies will focus on other endpoints, including reproduction and life span, to help with further elucidation of mechanistic pathway(s). This project is funded by Royal Thai Government.

TH047

The effect of silver nanoparticles on a freshwater alga (Pseudokirchneriella subcapitata)

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The nanotechnology industry has experienced rapid growth in recent years, with its products having diverse and novel applications across many fields. Despite this, the potential hazards associated with nanomaterials are still poorly understood, and as such there are as yet few regulatory guidelines which specifically address their safety. *Pseudokirchneriella subcaptitata* is a freshwater alga frequently used in standard toxicity tests, where its position at the base of the food web has a high ecological relevancy. Work done to date in this project has focussed on silver nanoparticle toxicity, due to its frequent occurrence in consumer products. The reference nanomaterial used was OECD NM300K, an aqueous dispersion of silver nanoparticles of a modal size of 15 nm (99% 3 was used to compare the effects of silver ions to their nanoparticulate form. Growth inhibition data (derived from the OECD 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test) were compared to photosystem II inhibition, as the later may represent a more practical estimate of toxicity due to smaller required volumes and shorter exposure times. Preliminary results suggest that test sensitivity is highly dependent on media choice and age of particles. Growth inhibition tests have shown higher toxicity in a non-standard medium (Jaworski's medium) compared to iron-enriched OECD medium (OECD-M medium). Growth inhibition and photosystem II inhibition both decreased with exposure time (up to 24 and 72 hours, respectively) in both media. This may be due to loss of nanoparticles to the test system, or the development of tolerance in *Pseudokirchneriella subcapitata*, and requires further investigation. Further work currently being planned includes other non-standard methods, including high throughput well-plate and flow cytometric analysis. The use of cell viability stains such as fluorescein diacetate, resazurin and propidium iodide will be investigated for their ability to detect sub-lethal effects such as enzyme activity and oxidative stress. Such methods may be of great practical utility to regulatory bodies due to higher replication and ease of sampling, compared to existing regulatory tests. Funding for this project was provided by the European Commission FP7 project MARINA (Managing the Risks of Nanomaterials), which seeks to develop and validate risk management methods for nanomaterials.

Applications of innovative passive sampling and dosing (P)

TH048

Development of passive samplers for the simultaneous measurement of inorganic mercury and methylmercury in surface waters

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Mercury is a metal naturally and widely present in the environment but human activities have greatly increased its concentration in all environmental compartments. This element, particularly under its organic form methylmercury (MeHg), is a global environmental concern due to its high toxicity, its capacity to bioaccumulate and to biomagnify in the food chain. In aquatic systems, mercury is generally present at low concentrations (sub-ng/L levels); hence the development of "ultra-clean" sampling techniques and the use of ultra-trace analytical techniques are necessary. Conventional techniques such as spot or automated sampling are generally time-consuming, do not take into account temporal variability and could induce samples contamination. To overcome these limitations, the Diffusive Gradient in Thin film (DGT) technique is an alternative technique to sample labile metals in surface waters (Davison and Zhang, 1994). DGT have been widely used to measure cationic metals especially those belonging to the priority substances list of the Water Framework Directive (WFD) such as Pb, Cd or Ni in natural waters, sediments or soils. However, classical DGT based on a polyacrylamide diffusive gel and a Chelex resin do not allow to sample inorganic mercury and MeHg, which is also a priority substance of the WFD. Consequently, the development of a specific diffusive gel and resin is necessary to measure mercury in waters. The aim of this study is to develop passive samplers coupled to gaz chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) for the simultaneous measurement of inorganic mercury Hg(II) and MeHg in surface waters. For that purpose, two types of DGT (home-made 3-mercaptopropyl functionalized silica gel and commercial spheron-thiol both combined with diffusive agarose gel) have been tested and compared under controlled laboratory conditions in spiked synthetic waters. Kinetic experiments show low level of blanks for the home-made resin compared to the commercial resin (DGT Research), as well as a good reproducibility (< 25%). Diffusion coefficients in the agarose gels have been jointly determined for Hg(II) and MeHg. Moreover, the inorganic mercury and methylmercury fractions trapped by DGT have been determined by using optimized GC-ICP-MS procedure after extraction and derivatization. *In situ* measurements will be carried out in a contaminated river from northern France (the Deûle) to detect both mercury species.

TH049

Monitoring of Cu and Cd in natural aquatic environments by using Diffusive Gradients in Thin Films technique - DGT modified with biopolymer.

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The in situ monitoring of trace metals in aquatic environments is crucial to the understanding of those physicochemical processes in the environment. The DGT technique allows monitoring through a passive sampler which uses Chelex-100 resin as a binder. In this work, a chitosan film, that is a natural biopolymer with significant adsorption of metals ions, was evaluated as a binder to the DGT for determination of Cu and Cd. The chitosan film was evaluated by Atomic Force Microscopy - AFM and Spectroscopy Fourier Transform Infrared - FTIR. The film interactions with metals were investigated using disks of 4.9 cm^2 and 50 mg of chitosan, in solutions containing 0.5 to 10 mg L^{-1} of metals, pH = 5 and I = 0, 01M.To DGT technical were determined elution factors (f) of metals by using HNO_3 solutions of 0.5, 1.0 and 2.0 M. Using DGT devices, immersion curves (adsorbed mass at different times) were made using solutions of 1 mg L^{-1} of Cd and Cu at pH = 5 and I = 0.01 M. The results of adsorbed masses were compared with their theoretical values. With the best results observed for Cu, the other tests were performed only with it. The effects of pH and ionic strength were observed in solutions containing 1 mg L^{-1} of Cu, pH ranging from 4 to 6 and I between 0.0008M to 1M. The influence of interfering ions Zn, Fe, K and Ca was observed. The DGT performance using chitosan film was verified in natural waters samples with spiked of 0.5 mg L^{-1} of Cu. The AFM images of films showed a granular surface but smooth. The comparison between FTIR spectra from powder and film of chitosan showed higher presence of hydroxyls in the film, derived from its hydration. The kinetic experiments showed high adsorption of the metals analyzed, was higher for Cu (90% at 500 min) and lower for Cd (45% at 150 min.). The elution of the analytes were more efficient with 1 M HNO_3 (f = 1 for Cu and 0.78 for Cd). For the tests with the DGT devices, the immersion curve of Cu was better with $R^2 = 0.9958$ and one difference of less than 10% theoretical. The influence of pH on the determination of Cu was small, with recovery of 90% for pH range between 4.0 and 6.0 and there were no significant differences in range I evaluated. The ion Fe showed greater interference in the adsorption of Cu, with reduced by 48%. For the determination of Cu in natural waters samples, the comparative results between commercial DGT device (Chelex-100) and the proposed device showed similar performance.

TH050

Measuring the free concentration of cationic surfactants – Application and validation of a combined SPE/SPME method and assessing the influence of sorption to glassware, septa, and pipette tips

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Cationic surfactants are a challenging but poorly studied group of environmental contaminants. One of the key difficulties when dealing with cationic surfactants is their tendency to accumulate on interfaces, which leads to losses during handling of aqueous samples. A previously developed SPE/SPME method was validated. The recovery for weak cation exchange (WCX) SPE cartridges was determined for a broad range of cationic surfactants, consisting of alkylated primary, secondary, tertiary and quaternary ammonium compounds, including benzalkonium and pyridinium structures. Affinity for 7 μm polyacrylate coated fibers was determined for these compounds; full sorption isotherms were measured for selected compounds. WCX-SPE was employed to verify the aqueous concentrations used to calculate fiber partitioning coefficients. Glass binding to clear glass and amber glass vials was also determined for the majority of compounds, as was binding to polyethylene pipette tips and PTFE, polyethylene or aluminum coated septa. All measurements were performed on an LC-MS/MS. Together, the data gathered in these experiments provide a good picture of the difficulties and several solutions when dealing with the analysis of cationic surfactants in aqueous samples.

TH051

A hydrophobicity parameter for surfactants: Partitioning of linear alkylbenzenesulfonate compounds to SPME fibers

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Anionic and nonionic surfactants are large-scale produced chemicals which can be found in many consumer products and consequently also in the environment as organic contaminants. Therefore, it is important to understand the fate and pathways of these compounds. Sorption to soil or bioaccumulation into organisms of these surfactants is mostly driven by the hydrophobicity of the individual compounds. Differences in hydrophobicity of organic compounds are usually expressed via octanol-water partition coefficients. However, this method is not applicable to surfactants because of their surface-active properties. In this study, solid-phase microextraction (SPME) fibers were used to examine partitioning of several anionic and nonionic surfactants to a hydrophobic phase. The compounds cover different surfactant groups, including alcohol ethoxylates, linear carboxylic acids, linear alkylsulfates, linear alkylsulfonates, linear alkylbenzenesulfonates (LAS), perfluorocarboxylic acids, and perfluoroalkylsulfonates. The data will be used to develop a new hydrophobicity parameter for the prediction of sorption, bioaccumulation, and toxicity of these surfactant groups. The current study focuses on the partitioning of LAS compounds between polyacrylate-coated SPME fibers and water, and the mechanisms that influence this partitioning. A method to determine partitioning of LAS compounds between fibers and water has been developed. The compounds are absorbed from the water phase by the fiber. After equilibration, the fiber is desorbed in an organic solvent and subsequently measured with liquid chromatography and fluorescence detection. The results show that the more hydrophobic LAS compounds show a higher uptake by the fiber. Furthermore, pH and salinity are important factors affecting the partition behavior of these surfactants. Therefore, this SPME method may be a suitable alternative of the octanol-water method in the case of LAS compounds. Further work will explore the possibility of using the fiber-water partition coefficient as a hydrophobicity parameter for other surfactant groups.

TH052

Partitioning of hydrophobic organic chemicals between silicone polymers and LDPE

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Hydrophobic organic chemicals (HOCs) are widely distributed in the environment. To monitor their occurrence and fate, and to determine their toxic potential, several methods exploit HOC partitioning using polymer-based passive sampling and dosing. A wide range of polymeric materials is available, and especially different silicone materials are often preferred for HOCs offering great flexibility in application formats. Differences between polymeric partitioning phases can be determined on the basis of polymer-polymer partitioning coefficients. Simple co-exposure experiments were thus conducted with a wide range of silicone materials and one low density polyethylene. The obtained polymer-polymer partition coefficients (1) revealed differences in the partitioning of HOCs between polymers, (2) indicated the presence of fillers in some polymers, (3) indicated differences in chemical structure of some silicone polymers and (4) allowed to determine the contribution of partitioning differences between polymers to the total variability in published polymer-water partition coefficients. Finally, we present strategies on how polymer-polymer partition coefficients can be applied for correction of differences between passive sampling and dosing materials, and for the determination of polymer-specific partition coefficients.

TH053

Development and performance testing of a mobile passive sampling concept for screening of organic pollutants in water

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Screening of trace organic compounds, their toxicological effects and associated risks in the water column of large rivers, lakes and seas is one of the potential future applications of passive samplers in routine investigative monitoring under Water Framework Directive. Passive samplers can accumulate pollutants in a similar way as organisms do and concentrate sufficient amounts of pollutants from water for chemical and toxicological analysis where conventional spot or bottle sampling methods often fail. We present an "active" passive sampling system (APS) for temporally and spatially integrative sampling of organic pollutants in situations when time to accumulate sufficient amount of pollutants for quantification is too short for application of classic passive sampling. APS can be used in a concept similar to that of a Ferry-Box to obtain a representative picture of pollution situation along defined stretches or transects of large water bodies including rivers, lakes or seas. To allow short sampling times the APS is designed to maximize uptake rates, which is achieved by sampling at elevated water flow velocity, 1-2m/s. For this purpose samplers are placed in a small flow-through chamber. A high flow pump is used to achieve the desired high flow needed to reduce the water boundary layer on surface of samplers. In contrast to a typical Ferry-Box there is a direct water intake to the exposure chamber with minimal auxiliary equipment (tubing) what minimises artifacts related to the undesired sorption of compounds. The uptake principle in the APS remains the same as in classical static passive sampling and the monitoring results can be evaluated using usual passive sampler calibration parameters. We tested the performance of the "active" passive sampling device against a static passive sampler by measuring sampler uptake kinetics up to 14 days in treated waste water from a large municipal treatment plant. We applied two types of passive samplers: a partitioning sampler, based on silicone rubber spiked with performance reference compounds, and an adsorption sampler, based on styrene-divinylbenzene solid phase extraction disks, respectively. Results for uptake of selected polar pesticides, organophosphate flame retardants, musks, polycyclic aromatic hydrocarbons, organochlorine pesticides and polychlorinated biphenyls will be presented and the feasibility for application of the device in ship surveys will be discussed.

TH054

Spatiotemporal variation of an urban lake chemical quality - interactions between organic matter and organic micropollutants

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In urban area, lakes are considered as sentinels and good indicators to estimate environmental changes due to their vulnerability and their capacity to drain a large urbanized catchment [1]. To better understand the ecosystem functioning of the lake, it is necessary to improve our knowledge about its chemical contamination and in particular about the spatiotemporal variations in relation to local perturbations such as stormwater discharges. In the aquatic environment, the fate and availability of organic micropollutants are related to their interactions with dissolved and particulate organic matter (OM) [2, 3]. To monitor these interactions *in situ*, integrative passive samplers based on polymeric membranes have been developed [4] and it is conventionally assumed that they sample the available part of micropollutants. The objectives of this work were (i) to adapt the methodology of polymeric membranes to the lakes; and (ii) to evaluate the contamination of chemical parameters: organic matter, polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs); and to assess the spatiotemporal variability of interactions between OM and organic micropollutants. Polymeric membranes in LDPE, doped with tracers, were exposed in 5 points of the Creteil Lake (in the middle of the water column at 3 points on a longitudinal transect from the stormwater discharge to the outlet of the lake, and in 3 depths at the central points) during 7 one month-periods. In parallel, monthly concentrations of organic matter and micropollutants were measured in the dissolved and particulate fractions using grab samples. The contamination of the stormwater discharges were also monitored during 6 storm events. The use of polymeric membranes has been validated for the specific hydrodynamic conditions of lacustrian environment and this tool seems promising as an alternative method to improve chemical monitoring of urban lake. Partition coefficients between the different fractions have been evaluated (K_d, K_{OC} and K_{DOC}). The spatial variability of contaminant concentrations is of the same order of magnitude as the analytical uncertainty showing that the lake can be considered spatially homogeneous for these parameters. The temporal monitoring suggests a decrease in dissolved and available dissolved fractions during the summer, which could be related to algal developments resulting in a change in quality and quantity of organic matter. The impact of stormwater discharge is being studied.

TH055

Estimation of POCIS field sampling rate values for selected pharmaceuticals

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There is a growing concern about the occurrence of pharmaceuticals and personal care products (PPCPs) in aquatic environment. Such compounds are not effectively removed by waste water treatment plants (WWTP). Therefore, PPCPs are detected in WWTP effluent, consequently reaching surface waters. Among the sampling methods, spot sampling is the most frequently used one. The main disadvantage is that the information obtained from the sample is unique to the place and the time selected. Passive sampling is a promising tool for the water monitoring with significant advantages over the commonly used techniques, being less sensitive to accidental variations of the pollutant concentration and gives time-weighted average (TWA) concentrations. Remaining issue for the use of POCIS is recalculation of concentrations. Environmental concentrations obtained from passive sampling can only be estimated when pre-calibrated sampling rates are used. Calibration experiments are usually made in laboratory conditions. It is important to conduct in situ evaluation to adjust laboratory calibrated sampling rates. Calibration experiment was carried out under the real conditions in surface water of the stream affected by wastewater treatment plant effluent. Standard pharmaceutical configuration of POCIS was used. POCIS were exposed for 14 days at four different locations along the stream in October, January and April. Simultaneously, water samples were taken. All water samples were analyzed by LC/LC-MS/MS method. The POCIS samples were analyzed after conventional extraction procedure by LC-MS/MS. Sampling rate values were calculated for representatives of pharmaceuticals from different groups and then compared with sampling rates from calibration experiment in effluent wastewater conducted earlier and also sampling rates available in the literature. Calculated sampling rates were also used to estimate water concentrations for water samples taken from the same stream. **Acknowledgements** This study was supported by CENAKVA CZ.1.05/2.1.00/01.0024, the Grant Agency of USB GAJU 087/2013/Z and the Czech Science Foundation GACR P503/11/113, the Ministry of Education, Youth and Sports of the Czech Republic Kontakt II LH12179, CZ.1.07/2.3.00/20.0024 Strengthening of Excellence scientific teams in USB FFPW, the results of the project LO1205 were obtained with a financial support from the MEYS of the CR under the NPU I program.

TH056

Use of passive samplers (POCIS and SPMD) for the evaluation of the efficiency of wastewater advanced treatments

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Our objective is to study some advanced processes, such as ozonation (O_3) or Granular Activated Carbon (GAC) as example, used in tertiary stage of wastewater treatment plants (WWTP). The challenges when studying such processes is to be able to measure organic micropollutants at very low concentrations (ng/L). To achieve this goal, passive samplers (PS) like POCIS (Polar Organic Chemical Integrative Sampler) and SPMD (Semi-Permeable Membrane Device) were used. The results of chemical analyses on PS were compared with those on grab water samples. Four one-month-long sampling campaigns were carried out in 3 different WWTP: 1) O_3 (full-scale) followed by GAC (pilot) in WWTP A, 2) O_3 only (full-scale) in the same WWTP A, 3) GAC (pilot) in WWTP B, 4) polishing pond (full-scale) in WWTP C. In all campaigns, POCIS were exposed in water during 14 days and SPMD during 28 days. To be able to compare these 4 campaigns and the molecule concentrations upstream and downstream the tertiary treatment, POCIS and SPMD were always immersed in an aquarium in the same controlled experimental conditions of flow (280 mL/min), temperature (20° C) and light. To insure quality of data, POCIS and SPMD were exposed in triplicate and field blanks were realized (i.e. PS exposed to the ambient air). In parallel, 2h composite water samples were collected at D0, D14 and D28. Targeted chemical analyses were performed on POCIS extracts for 117 hydrophilic compounds belonging to pharmaceutical, hormone, pesticide and alkylphenol classes and on SPMD extracts for 46 lipophilic compounds belonging to chlorine pesticides, Polycyclic Aromatic Hydrocarbon, PolyChloroBiphenyl, PolyBrominated DiphenylEthers classes. Hydrophilic compounds and PAH were also analysed in water samples. We will present results obtained for SPMD and POCIS. As an example, compared to grab sampling, POCIS allowed detecting 7 more pharmaceuticals in influent of tertiary treatments and 4 more in effluents in the 2 first sampling campaigns. Nevertheless, information on process efficiency is the same since the decrease of pharmaceutical concentrations after advanced water treatment is in the same order of magnitude with grab and passive sampling in the 2 first sampling campaigns. Hence, POCIS appears as an interesting tool to characterize tertiary stages of WWTPs with analysis of organic micropollutants at very low concentrations.

TH057

Calibration and field evaluation of passive samplers for monitoring pesticides in water

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Passive sampling is a promising tool for monitoring of pesticides in water with minimal infrastructure and detection of contaminants at low, environmentally relevant concentrations. Passive sampling is based on an in-situ deployment of devices/sorbent capable of accumulating contaminants freely dissolved in water. Such accumulation occurs via diffusion, typically over periods of days to weeks, and can be described by the compound specific sampling rate, which is the equivalent volume of water accumulated by the sampler per unit of time. The continuous emissions of pesticides to the aquatic environment are posing a risk to wildlife and human health. In this study, passive samplers were characterized for over 100 individual pesticides in the water phase. In addition, passive samplers were applied in two Swedish river systems and the concentration was compared to active sampling. Sampling rates and polymer-water partitioning coefficients for five different passive sampler types will be presented including *i)* polar organic chemical integrative sampler (POCIS)-A (Oasis hydrophilic–lipophilic balance (HLB) sorbent), *ii)* POCIS-B (triphasic sorbent admixture, Isolute ENV+ and Ambersorb 1500), *iii)* silicone rubber, *iv)* Chemcatcher® SDB-RPS (styrene divinyl benzene Empore™ disk), and *v)* Chemcatcher® C18 (Empore™). disk. Overall, the results of this study will improve our understanding of the concept, challenges and application of passive sampling for future monitoring strategies of a broad range of pesticides in water.

TH058

International Pellet Watch : Distribution of POPs and emerging chemicals in microplastics

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International Pellet Watch (IPW) is global monitoring of hydrophobic organic pollutants by using plastic resin pellets. IPW provides information of status and trend of marine pollution. In addition, IPW gives us basic information of chemical risk associated with marine plastics. Since 2005, we have analyzed pellets from ~ 150 locations from ~ 40 countries for PCBs, DDTs, and HCHs. Hot spots of PCB pollution were identified, e.g., Le Havre (France), Athens (Greece), Tokyo Bay (Japan), Los Angeles (USA). IPW combined with passive air samples (PAS) indicates that legacy pollution is major contributor to most of the hot spots of PCB pollution. However, current inputs of PCBs were identified in some areas such as Manila Bay, Philippines and Greater Accra Region, Ghana where contribution from e-waste was suggested. Higher concentrations of HCHs derived from Lindane were observed in Southern hemisphere (Australia, New Zealand, Mozambique, South Africa) than Northern Hemisphere, suggesting current usage. This is consistent with spatial pattern of HCH pollution of seabirds. We analyzed a pellet sample collected in 2013 from an island of Mozambique where high concentration of HCHs (36 ng/g) was recorded in a pellet sample collected in 2007. The sample collected in 2013 showed trace concentration of HCHs (0.3 ng/g), suggesting effectiveness of regulation of HCHs by Stockholm Convention as from 2008. In addition, toxic chemicals unregulated by Stockholm Convention, including emerging chemicals, were analyzed in the present study. Polycyclic aromatic hydrocarbons (PAHs) were analyzed in pellet samples from ~ 30 locations around the world. PAH concentrations (sum of 28 compounds) ranged from limit of quantification (LOQ; < ~ 100 ng/g) to 24000 ng/g-pellet. At most of the locations, PAH concentrations were below or close to LOQ. This might be due to photo-degradation of PAHs during floating on sea surface and stranding on beaches. However, extremely high concentrations of PAHs were sporadically observed at several locations and they were abundant in methylated PAHs. These petrogenic PAHs are likely to be derived from local oil spills. Furthermore, triclosan and methyltriclosan were detected in the pellet samples with concentration range from 1 ng/g to 62 ng/g and 1 ng/g to 69 ng/g, respectively. Their distributions were associated with sewage inputs.

TH059

Passive samplers as a means to monitor urban biocide emissions

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Biocides emerging from facades antifouling paint have recently been discussed as a

pressure for surface waters in urban environments. Monitoring of the immission situation and compound profiles are cumbersome due to the dynamic occurrence of the biocides during rain events mainly: campaigns with triggered autosamplers are currently the state of the art monitoring technique. For river basin managers which have to mitigate emissions from built surfaces to receiving waters it is important to gain an overview of the current pollutant profiles and loads. Autosamplers are too work-intensive and costly to be used at larger scale. Passive samplers can be an interesting alternative once the quantitative nature of their sampling behaviour under the relevant hydrological conditions has been established. We have shown in recent work that the pesticide surface runoff events in catchments can be calculated from passive sampler records over longer periods. Although only compounds with log Kow > 1 allow for the time-proportional reconstruction of mean event concentrations, the method proved to be robust compared to autosampler data. Since the most prominent biocides used in antifouling paint of facades are parented to agricultural herbicides it seemed logical to test passive samplers for immission monitoring in rivers. For that purpose an autosampler has been run in parallel to passive sampler exposure during precipitation events in an urban catchment with substantial sewer overflow contributions and urban runoff. This allowed for comparing the pertinence of sampling rates for passive samplers from the literature or laboratory experiments. On the other hand passive samplers have been exposed in 14 different catchments with varying degrees of urbanisation and surface runoff management (combined vs. separate sewer systems). The results showed which compounds were most prominent, exposure peaks and dynamics as well as the link to the urban layout.

TH060

Emerging pollutants detected in passive samplers exposed in middle reaches of the Yangtze River

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In this project, we aim to assess the potential occurrence and impact of biologically active substances on aquatic environment of the Yangtze River, mainly focusing on emerging pollutants. The project is focused on identification and screening of anthropogenic pollution in middle and lower reaches downstream the Three Gorges Reservoir. The special interest of this project is to detect and identify relevant emerging pollutants using passive sampler/mass-spectrometry approach, with specific attention to persistent organic pollutants (POPs), biocides, pharmaceuticals and personal care products (PPCP). This study explores use of integrative passive samplers followed by a novel analytical approach LC-HRMS and GC/GC-TOF HRMS, which can provide additional valuable data to conventional monitoring program. Pilot sampling of the Yangtze River was done during September 2012. Based on results of pilot sampling, the exposure period was optimized for consequent sampling of 10 localities in 2013. Following contaminants were measured: pharmaceuticals (118), pesticides (52), perfluorinated acids and sulfonates (19), drugs and metabolites (23), UV blockers (4). Several contaminats show an increasing trend downstream from the Three Gorges Reservoir towards the last sampling site in WuXue . Acknowledgement: This study is supported by the MŠMT CR project Kontakt II - LH 12179, CENAKVA CZ.1.05/2.1.00/01.0024 and the Grant Agency of USB GAJU 087/2013/Z. The results of the project LO1205 were obtained with a financial support from the MEYS of the CR under the NPU I program.

TH061

Concentrations, Fluxes, and Fate of Polycyclic Aromatic Hydrocarbons of the Yellow River during Water-Sediment Regulation of the Xiaolangdi Reservoir

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Water-sediment regulation of the Xiaolangdi Reservoir is to control the relationship between riverine runoff and sediment transport of the Yellow River; however, there is no research report about its effect on pollutant transportation and flux. In this research, the water and sediment samples were collected downstream the Xiaolangdi Reservoir from June 21 to July 10 (2013) during the water-sediment regulation. The river runoff, suspended sediment (SPS) concentration, and the concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) in different phases were determined. In this research, the freely dissolved concentrations of PAHs were measured by polyethylene devices (PEDs) in situ, and the PEDs were preloaded by deuterated PAHs in order to shorten their exposure time during field deployment. Both the total and freely dissolved concentrations of most PAHs during water regulation were lower than those during sediment regulation. This was due to that the resuspended sediment content during sediment regulation was much higher than that during water regulation, which made more contaminants release from the sediment. During the sediment-water regulation, the $\log K_{DOC}$ values of phenanthrene, pyrene and chrysene in river water were 2.39-2.60, 2.78-3.08, and 3.22-4.32, respectively, which were significantly negatively correlated with the $\log K_{ow}$ values of PAHs ($p < 0.05$). In addition, the fluxes of water and sediment into the Bohai Sea were 5.245 billion m^3 and 56.1 million tons, respectively, during water-sediment regulation. The flux of Σ_{16}PAHs was 11.41 tons, including Σ_{16}PAHs in particulate phase (10.89 tons) and dissolved phase (0.52 tons). Because of the large flux of total PAHs and the release of PAHs from resuspended sediment during water-sediment regulation, the effect of water-sediment regulation on the bioavailability and ecological risk of PAHs and other contaminants to aquatic organisms should also be considered in the Xiaolangdi Reservoir management in the future.

TH062

Latin American Atmospheric Passive Sampling Network (LAPAN): First outcomes

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After 3 years of operation, the Latin American Atmospheric Passive Sampling Network (LAPAN) has XAD2-based samplers installed at 58 sites covering areas of different backgrounds (industrial, rural, urban and non-impacted areas) at 8 different countries. This network was established to improve the research of atmospheric contaminants within Latin America. Local, regional and global sources and spatial and temporal distribution of POPs, among other contaminants, are being assessed. This study will enable signatory countries to comply with Stockholm Convention demands on the identification of main sources and provide monitoring comparable data on POPs contamination. This will help to identify areas of concern and to evaluate the effectiveness of the control actions adopted by the member countries to eliminate the release of POPs to the environment. The XAD2-based passive atmospheric samplers has the advantage of being cheap and easy to run, making it possible to increase the present spatial and temporal resolution run by GAPS (*Global Atmospheric Passive Sampling Network*) and requested by the Guidance on the Global Monitoring Plan (GMP) for POPs. As a result, LAPAN is now a consolidated network which significantly enhanced the spatial and temporal resolution in a sustainable manner to cope with long-term studies. The regional capacity building has been improved and is continuously being addressed by the network members. Some of LAPAN results (mainly background sites) were incorporated in the second Global Monitoring Report, which will be presented for the 2014 Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants. Among other POPs, endosulfans (the parental endosulfan predominated) were the main contaminant in air of South America.

TH063

Application and calibration of XAD-2 passive samplers for the evaluation of the atmospheric contamination by current-used pesticides and their variability

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Actually, methods used to evaluate the atmospheric contamination by pesticides use high volume samplers followed by solvent extraction, purification and analysis by chromatography. This technique is well known and efficient but presents some disadvantages, especially when sampling campaigns are generated in a large scale and for a long time for example. Indeed, samplers need electrical connexion (not easy on the field) and time consuminglaboratory practices. An alternative to this method is the use of passive samplers which are inexpensive and easy to use and to deploy in a large scale. If the concept and theory of passive sampling are well known, many works need to be performed in fundamental or practical points of vue, in particular on extraction and purification processes after sampling and on the applicability of passive sampling of polar compounds. Indeed, in the actual literature, only lipophilic compounds are of concern, probably due to their easiness of extraction and analysis. Passive samplers which were developed in this study used XAD-2 put on meshed Inox tubes with dimensions compatible with 100 mL ASE cells in order to permit the direct introduction of the sampler for extraction

after sampling. Extraction was done with acetonitrile. The obtained solvent was concentrated to 1 mL using a rotatory evaporator, and the residual was diluted with water to allow analysis by SPME coupled to GC-MSMS. With this method, an increase of sensitivity was obtained, and no further concentration step was needed. Passive samplers were deployed during pesticides application periods in urban (Strasbourg) and rural (Sand) area in east ofFrance, and sampling was done on 15 days periods from 2011 to2012. Inparallel, active sampling on filters followed by XAD-2 resins was performed during the exposure period of PAS in theStrasbourgsite in order to obtained data for tentatively XAD-2 PAS calibration. Eight passive samplers were also exposed together for between April and June 2012 and of PAS was removed each week in order to evaluate the accumulation potential of XAD-2 PAS. Pesticides detections are in accordance with applications on crops inAlsaceand shows an important seasonal trend as already observed for current-used pesticides by active sampling. Details of the detection of pesticides and of variability in space and time together with calibration from field campaign and accumulation potential will be discussed in the poster.

TH064

PASSIVE AIR SAMPLING OF POPs ACROSS ASIA: POLYBROMINATED DIPHENYL ETHERS (PBDEs)

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Passive sampling in the environmental research is getting an increasing attention as an alternative to the expensive and more laborious active sampling. It can provide time-integrated samples for air and water. In this study, the contamination status and characteristics of PBDEs in Asian atmosphere were using passive air sampler (PAS). Polyurethane foam (PUF)-type PASs were deployed for 28-95 days (every year from June to October, 2010-2012) at urban and rural at 11 locations from 9 countries (Korea, China, Vietnam, Thailand, Philippines, Malaysia, Indonesia, India and Sri Lanka). The mean concentrations of Σ_9PBDE (defined as the sum of BDE-17, -28, -47, -66, -100, -99, -154, -153, and -183) in Asian urban and rural area were 19.5±6.5 pg m^{-3} and 16.4±7.5 pg m^{-3}, respectively. The Σ_9PBDE was relatively higher in urban areas than that of rural. The mean concentrations of PCBs (Urban: 175 pg m^{-3}, Rural: 45.8 pg m^{-3}) and DDTs (Urban: 158 pg m^{-3}, Rural: 89.3 pg m^{-3}) were also found to be on the same trend like Σ_9PBDE. Thehighest level of Σ_9PBDE was detected in the rural sites of Beijing, China (mean: 34.6 ± 37.4 pg m^{-3}) followed by urban sites of Indonesia (28.1 ± 10.9 pg m^{-3}) and Malaysia (26.1 ± 16.5 pg m^{-3}). Although Korea is one of the biggest markets of flame retardant, accounting for about 10% of global use, the Σ_9PBDE concentration was relatively low in Korea compared to other Asian countries. Considering Deca-BDE accounts for the major portion (99%) of commercial PBDE mixtures in the Korean market, the relatively low level of Σ_9PBDE in the Korean air might be due to no inclusion of BDE-209 for the calculation of Σ_9PBDE. If the amount of BDE-209 retained in each PUF disk was compared, Korean sample would result in the highest value among Asian countries and followed by Beijing, China. In fact, BDE 209 was predominant BDE congener in both urban and rural samples from Korea and China, indicating the abundant use of Deca-BDE. Lower brominated congeners such as BDE-47 and BDE-99 were dominant in samples from Indonesia and Malaysia. Therefore, for the comprehensive understanding of PBDE pollution in atmosphere using PAS, BDE-209 should be considered and discussed.

TH065

Passive Equilibrium Sampling of Hydrophobic Organic Contaminants in Sediment

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Hydrophobic organic contaminants (HOCs) reaching the aquatic environment are largely stored in sediments. The exposure risk of contaminated sediments is challenging to assess since traditional exhaustive extraction methods capture both the freely dissolved and the bound fractions. Contrarily, only the freely dissolved concentration (C_{free}) represents the bioavailable fraction and effective concentration for diffusive uptake and partitioning. Passive equilibrium sampling approaches can deliver C_{free}: A range of silicone-based formats have been described, e.g. glass jars with μm-thin silicone coatings on the inner vertical walls for equilibration in the laboratory [1] and a device housing a number of silicone-coated fibers for *in situ* equilibration [2]. In both cases, the parallel sampling with varying silicone thicknesses can be applied to indicate valid equilibrium sampling, avoiding tedious time series measurements. The measured equilibrium partitioning concentrations in the silicone (C_{Sil}) can be used directly, e.g. for the assessment of spatial trends or comparison with C_{Sil} from other media such as biota [3]. Furthermore, C_{Sil} can be divided by silicone/water partition ratios [4] to yield C_{free}. In order to assess HOC levels in biota, C_{free} can be multiplied with bioconcentration factors (BCFs) to predict concentrations in benthic organisms [5], but the uncertainties in BCF determination also apply to these predictions. A more accurate approach is to calculate concentrations in model lipids at thermodynamic equilibrium with the sediment ($C_{Sed,lip}$) as the product of C_{Sil} and lipid/silicone partition ratios [6], which has been done in several studies with limnic and marine sediments [3,7-8]. The $C_{Sed,lip}$ data can then be compared with lipid-normalized concentrations in aquatic organisms or regulatory thresholds. Silicone-based passive equilibrium sampling has proven to be a straightforward, precise and sensitive approach to determine the effective concentrations of HOCs relevant in risk assessment that may be useful for sediment management decision-making. **References**. [1] Reichenberg et al. *Chem. Cent. J.* **2008** 2:8; [2] Witt et al. *Environ. Sci. Technol.* **2013** 47:7830; [3] Jahnke et al. *under revision*; [4] Smedes et al. *Environ. Sci. Technol.* **2009** 43:7047; [5] Kraaij et al. *Environ. Sci. Technol.* **2003** 37:268; [6] Jahnke et al. *Chemosphere* **2008** 73:1575; [7] Mäenpää et al. *Environ. Sci. Technol.* **2011** 45:1041; [8] Jahnke et al. *Environ. Sci. Technol.* **2012** 46:10114.

TH066

Concentration generator: a partition controlled delivery system to continuously solubilise highly hydrophobic, volatile and biodegradable substances for ecotoxicological test.

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Having access to true water solution of targeted substance is key to conduct (eco)-toxicological tests. When dealing with highly hydrophobic (logP>4), biodegradable and volatile substances, the challenge may become really tricky. Use of cosolvent or surfactant may help but can also impact the test results, and as such should be avoided. Muscenone delta, a macrocyclic musk extensively used in perfumery applications, is typical from such substances. Use of cosolvent was demonstrated to be unable to generate a true solution of this low soluble product (solubility= 0.9mg/L). We describe here a simple way to control the enrichment of water with a hydrophobic compound by diffusion from a polymeric matrix. Based on passive diffusion through a PDMS tube, the simple device that we call Concentration Generator is able to generate continuously a 100mL/min flow of a true solution of muscenone delta during more than 10 days. By varying the length and thickness of the tubing, the concentration can be adjusted to fit the requirements of the test. The concentration was proven to be stable over the entire period of the test (variability < 20%, concentration ranging from 0.030mg/L to 0.500 mg/L), and the device has been used to conduct OECD 212 test. Different theoretical and practical aspects are discussed.

TH067

Soil to atmosphere PAH fugacity gradients in an urban lot measured by polyethylene passive samplers

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Soil-air exchange is a critical process controlling the fate of and exposure to persistent organic pollutants in the environment, such as polycyclic aromatic hydrocarbons (PAHs). To describe this exchange on a local scale, polyethylene (PE) passive samplers (50 µm thickness) were spiked with performance reference compounds (PRCs) (deuterated fluorene (d-FLU), pyrene (d-PYR) and benz[a]anthracene (d-BaA)) and deployed in UFO shields typical in passive air sampling at two different heights above the soil in a grassy lot off a city street in Tübingen, Germany once in spring and in summer. A vertical soil profile was also collected about 1 m from the sampler station for measuring both available PAHs via PE-soil batch tests and total concentrations via extraction by accelerated solvent extraction (ASE). Compounds that did not attain equilibrium over the deployment period were corrected with their respective k_es to estimate their vapor phase concentrations. Loss of d-FLU from PE followed first order kinetics so the k_e values for d-FLU were used to approximate the k_es of the target PAHs by applying a double film diffusion model. Loss of d-PYR and d-BaA was not consistent and the magnitude of their estimated loss rates as well as their relationship with one another are evidence that photodegradation contributing to the estimated loss rates. Total quantifiable PAHs in air calculated from PE concentrations in the spring (Sum_{15} PAHs of 143 and 168 ng m^{-3} respectively, at 0.2 and 2 m) were similar to summer deployment (Sum_8PAHs of 123 ng m^{-3} and Sum_{10} PAHs of 120 ng m^{-3} respectively, at 0.1 and 2 m). Considering measurement error, a gradient in the free atmospheric PAH concentration from these heights was difficult to distinguish. However, it was clear based on soil PE batch tests that a higher PAH fugacity lay in the soil, identifying net volatilization from soil to the atmosphere to dominate during the deployment periods at this urban site even in the vicinity of traffic sources.

TH068

Can solid-phase microextraction fibers be used to predict the hydrophobicity of non-ionic and anionic surfactants?

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Octanol-water partition coefficients (K_{ow}) are often used in environmental risk assessment as a simple measure for hydrophobicity of organic contaminants to predict bioaccumulation, toxicity, and sorption to soil. However, experimental determination of K_{ow} values for surface-active chemicals is not feasible. Surfactants are complex and often ionized molecules and environmental distribution of these compounds depends on solution chemistry variables such as pH and ionic strength. Standardized OECD methods to determine K_{ow} values are not considered applicable to surfactants, because surfactants accumulate at interfaces and emulsify both octanol and water phases. In addition, prediction of K_{ow} values by computational methods can produce meaningless results, because these methods are not designed to estimate properties of (charged) surfactants. An alternative approach to determine a hydrophobicity parameter for surfactants is therefore required. Polymer-coated solid-phase microextraction (SPME) fibers can be successfully applied to determine the hydrophobicity of surfactants. Fiber-water partition coefficients can subsequently be used to predict the environmental distribution of surfactants. Advantages of this method are that differences in pH and ionic strength are taken into account and phase separation is not required, because freely dissolved concentrations are directly determined by the SPME fibers. The applicability of SPME fibers to determine the hydrophobicity of non-ionic and anionic surfactants and to predict their bioaccumulation in aquatic organisms is explored in this study based on literature and experimental data from different surfactant groups. The possibility of using SPME fibers as a hydrophobicity parameter has important consequences for the regulatory evaluation of surfactants within REACH and OSPAR.

TH069

Effects of persistent organic pollutants on marine primary production: an experimental approach

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Although, the anthropogenic pressure on the marine environment has increased during the last six decades [1], the effects of hazardous chemicals on marine primary production remain unknown [2]. Phytoplankton dynamics are mainly driven by bottom up mechanisms such as solar radiation and nutrients and top-down control by zooplankton grazing, but it can be questioned whether persistent organic pollutants (POPs) should be seen as an additional driver determining phytoplankton dynamics. In this research, growth and biomass production of the marine diatom *Phaeodactylum tricornutum* was studied using a full factorial design with the following factors: nutrient regime (saturated regime (14 μmol $P.L^{-1}$ & 588 μmol $N.L^{-1}$), winter regime (2 μmol $P.L^{-1}$ & 60 μmol $N.L^{-1}$) and summer regime (0.5 μmol $P.L^{-1}$ & 30 μmol $N.L^{-1}$)), water temperature (10°C and 20°C) and POP exposure (control and ambient concentrations). Passive samplers were used to achieve exposure to ambient POP concentrations [3] after a 6 week period of pre-loading along the Belgian coast. As expected, the growth rate of *Phaeodactylum tricornutum* was higher at 20°C than at 10°C, and nutrient limitation reduced the carrying capacity of the algal populations. Furthermore, the sensitivity of *Phaeodactylum tricornutum* to the POPs depended on the nutrient availability. At a saturated nutrient regime the POPs had no influence on the growth of *Phaeodactylum tricornutum*. At a nutrient regime relevant for winter conditions, a POP-induced growth inhibition was observed at both temperatures. However, at a nutrient regime relevant for summer conditions, a modest POP-induced growth stimulation was observed at both temperatures. More research is needed to confirm the preliminary results that are reported here. [1] Dachs J, Mejanelle L. 2010. Organic Pollutants in Coastal Waters, Sediments, and Biota: A Relevant Driver for Ecosystems During the Anthropocene? Estuar Coast 33: 1-14. [2] Rockström J, Steffen W, et al. 2009. A safe operating space for humanity. Nature 461: 472-475. [3] Claessens M. 2013. The use of passive samplers as a central tool in integrated environmental risk assessments. Ghent, Belgium: Ghent University.

TH070

How do desorption kinetics of lipophilic compounds sorbed to suspended particles affect toxicity?

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Bioavailability of sorbed compounds is an ever debatable topic, which has been further accentuated during the recent years research on nano-particles and nano-particle facilitated uptake. Studies using passive samplers in natural waters and sediments have generally shown a good correlation between the SPME extractable fraction of lipophilic compounds and toxicity towards *Daphnia magna*. But is it the freely dissolved concentrations measured by SMPE that solely causes the toxicity, or can compound bound to food items or loosely sorbed to mineral particles still be bioavailable? And does the particle size that the compounds are sorbed to matter, as suggested by some of the recent research in nano-science? We investigate these questions in a passive-dosing/passive-sampling test system using well defined low concentration (25 mg/L) sorbents such as algae, clay materials, chalk, humic acid, charcoal, quarts etc suspended in media. By measuring sorption and desorption kinetics, both sorption capacity and the relative binding strength of the compounds to the sorbents can be determined. The compounds used are C^{14}-labelled propiconazole ($logK_{ow}$= 3.72) and bifenthrin ($logK_{ow}$= >6). The corresponding toxicity of the sorbed compounds relative to freely dissolved compounds is tested on *D. magna*, as its filter feeding behaviour makes it very exposed to particle bound pesticides.

TH071

Passive dosing as a tool to derive fugacity capacities of a variety of leaves for semi-volatile persistent organic contaminants.

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The silicone polydimethylsiloxane (PDMS) has proven useful as a tool in experimental environmental chemistry: It has been applied successfully as a passive sampler[1] as well as a reliable donor in passive dosing experiments[2]. In this study we extend the application of PDMS as a passive dosing source to derive fugacity capacities (*Z*) of a variety of matrices for semi-volatile persistent organic contaminants. *Z*, expressed in mol m^{-3} Pa^{-1}, is a measure of the extent to which a chemical can be absorbed by a given matrix at a specific temperature. It is an important and frequently used parameter in modeling the environmental fate of chemicals[3]. Our experimental apparatus, based on Mayer et al.[4], consists of a thin disc of the sample wedged between 2 layers of PDMS loaded with the chemicals of interest. The PDMS is left to equilibrate with the sample and based on the known *Z* values of the PDMS for these chemicals, the *Z* value of the sample can be derived. The ease of the setup makes the approach suitable to measure the *Z* values of a wide variety of matrices. The challenge lies in keeping the PDMS the dominant reservoir of the chemical in the system. Analyzing tissues that are too rich in lipid risks depleting the PDMS to the extent that it becomes hard to accurately determine the fugacity capacity. Within the scope of our studies focusing on the so-called 'forest filter effect' we have applied the method to a variety of leaves. Existing multimedia chemical fate models that incorporate a forest compartment currently only assign two different Z values for foliage: One for conifers one for deciduous trees[5]. The composition of leaves is highly variable however. By measuring *Z* for a wide variety of foliage and linking these values to species-specific properties, we hope to achieve a better understanding of the cycling of semi-volatile persistent organic chemicals in forest systems. New *Z* values can easily be incorporated into existing fugacity based models or translated to leaf/air partitioning ratios.

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TH072

Passive dosing improve chemical bioavailability and toxicity assessment of alkylphenols in fish in vitro bioassays

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Nominal dosing is the most commonly used approach to expose *in vitro* systems, where all of the theoretical chemical concentration is assumed to be bioavailable for the bioassay. *In vitro* exposures with hydrophobic chemicals have however shown a lower toxicity than expected compared to *in vivo* data, creating doubts whether nominal dosing approaches are sufficiently to ensure bioavailability of test compounds in the *in vitro* bioassays. It has previously been shown that chemicals with hydrophobic properties (Log Kow >3) bind to plate walls, seal and media components thus effectively reduce the the chemical`s bioavailability. In addition to the chemical`s decreased bioavailability it is also metabolized by/in the *in vitro* system, resulting in a further decrease of the chemical`s free fraction, potentially underestimating the chemical`s effect concentration. Compensating for such chemical loss may be performed through use of a partition-driven chemical dosing technique called passive dosing. The dosing method consists of a chemically spiked

non-biologically active polymer, such as silicone which continuously partition the chemical out into the media until it reaches equilibrium. Passive dosing has shown to be a promising *in vitro* dosing method when evaluated for various compounds. However, passive dosing is still under development for *in vitro* use and further knowledge about the chemicals partitioning behavior in different exposure medias and bioassays are required. The aim of this study was therefore to evaluate the alkylphenols (Log K_{ow} 3.1-5.8) partitioning behavior in different exposure solutions and media, and determine how passive dosing may be utilized for MilliQ H_2O toxicity assessment in a primary rainbow trout (*Oncorhynchus mykiss*) hepatocyte culture. The results showed a linear relationship between the partitioning co-efficient and the chemicals Log K_{ow} for pure water in the logK_{ow} range of 3.1-5.8, but this relationship was only observed in a limited range of LogK_{ow} for cell culture media (Log K_{ow} < 4). The results indicate that partition of hydrophobic alkylphenols are limited by unknown constituents of the cell growth media and illustrate the importance of characterizing partitioning behavior of chemicals in *in vitro* bioassays to improve the applicability of such methods as alternatives to *in vivo* testing approaches.

TH073
Exposure control of hydrophobic compounds in the Microtox® assay: comparing solvent spiking versus passive dosing
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In recent years, various passive dosing approaches have been developed to control exposure in toxicity bioassays. Establishing and maintaining stable exposure concentrations of hydrophobic organic compounds (HOCs) in the water phase is difficult because of their hydrophobicity leading to sorption and volatilization losses. This results in undefined and decreasing exposure, making interpretation of the results difficult. The Microtox® test system is a widely used assay for determining the general toxicity of chemicals. In this simple system, the luminescene inhibition of *Vibrio fischeri* in response to chemical stress is measured over short time periods. As part of a larger study looking into the applicability of Microtox® for studying mixture toxicity, solvent spiking was compared to passive dosing using silicone disks for introducing HOCs into the Microtox® assay. The effective concentrations causing 50% inhibition of luminescence (EC_{50}) were consistently lower for passive dosing compared to spiking. For acenaphthene, the passive dosing EC_{50} was 190 $\mu g\ L^{-1}$ compared to 402 $\mu g\ L^{-1}$ for spiking, i.e., these differed by a factor of two. For phenanthrene these differed by a factor of 15, with a passive dosing EC_{50} of 10.7 $\mu g\ L^{-1}$ compared to 170 $\mu g\ L^{-1}$ for spiking. Finally, a clear positive relationship with concentration was seen with increasing fluoranthene concentrations only with passive dosing, but full inhibition was not observed even at aqueous solubility. Therefore, even in this simple and short-term test, careful control of HOC exposure using an approach such as passive dosing is necessary. This is particularly important for mixture testing – as reflected in binary HOC mixtures giving a very limited and variable toxicity when spiking but higher toxicity with passive dosing.

TH074
Passive dosing of polycyclic aromatic hydrocarbons in the marine algae test using silicone O-rings
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Testing hydrophobic organic compounds (HOCs) in aquatic toxicity tests is difficult due to compound losses through volatilization, sorption to the test vessel and culture medium constituents. This results in poorly defined exposure, the bioavailable concentration is reduced and concentration-effect-relation might be underestimated. Passive dosing can overcome these problems by the continual partitioning of HOCs from a dominating reservoir loaded in a biologically inert polymer such as silicone. This procedure provides defined and constant freely dissolved concentrations and eliminates spiking with cosolvents. Passive dosing using silicone O-rings and PAHs as test substances was applied in the marine algal growth inhibition test with *Phaeodactylum tricornutum* (based on ISO EN 10253)in 24-well microtiter plates. The passive dosing material was loaded by partitioning from a methanol suspension of the respective compound (1). The O-rings were added to the wells at the beginning of the test. Agitation of the plates speeds up the release from the O-rings. The single toxicity of a range of PAHs was investigated in dilution series of O-rings up to their aqueous solubility in seawater. The concentration-dependent growth inhibition of *Phaeodactylum tricornutum* was then compared for passive dosing and standard spiking according to the standard marine algae test procedure on microplates. A comparison of the EC_{50} values of passive dosing vs. EC_{50} values of standard dosing showed an underestimation of the effects when using nominal standard dosing probably due to sorptive losses and limiting dissolution kinetics. Furthermore, passive dosing concentration-response curves were more reproducible and shifted towards lower concentrations by several orders of magnitude. Results show that the response is clearly not only dependent on the potency of the compounds, but also on its supply, sorption and consumption during the assay. Passive dosing is a practical and economical way of improving the exposure of HOCs in aquatic toxicity tests like the algae growth inhibition test or in bioconcentration tests. (1) K. E.C. Smith, N. Domb, R. Blust, P. Mayer (2010) Controlling and maintaining exposure of hydrophobic organic compounds in aquatic toxicity tests by passive dosing. Aquatic Toxicology 98, 15–24.

TH075
Changes In Gene Expression Under The Controlled Exposure Using Passive Dosing
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In order to apply sensitive molecular-level biomarkers to the evaluation of environmental risks, it is necessary to establish a quantitative dose-response relationship. Recently, passive dosing is regarded to be a promising new technique that provides a constant exposure condition of hydrophobic contaminants in the assay medium. The main goals of the present study were 1) to provide a quantitative comparison of the gene expression results obtained from the passive dosing method and the conventional spiking method and 2) to investigate the changes in gene expression with respect to the freely dissolved concentration and the exposure duration using passive dosing. Chlorpyrifos (CP), which is known to be oxidized by the CYP450 monooxygenases, was selected as the model chemical, and cytochrome p450 family protein 35A gene series (cyp35a1-5) extracted from a soil nematode, *Caenorhabditis elegans,* was analyzed using a real-time PCR. Freely dissolved concentration of CP rapidly decreased when the spiking method was used, and the expression patterns of cyp family genes varied greatly with the volume of exposure medium and the exposure duration. In contrast, the concentration in the assay medium was stable until the end of experiment when the passive dosing method was used. In addition, the level of gene expression increased linearly both with the exposure time up to 8 h and with increasing concentration. The observed increased gene expression at low concentration of CP could be explained by increasing body residue concentration of CP with exposure time. In conclusion, quantitative dose-response relationships for gene expression biomarkers could be derived when the constant exposure condition is provided and freely dissolved concentration is used as the dose-metric.

TH076
From the initial monitoring to the development of a reference method. A case study on pharmaceuticals residues on the Paris area.
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Concern regarding the presence of potentially active substances in surface waters was brought up by the end of the 90s. Intensive monitoring programs have revealed a chronic state of contamination of water systems by numerous molecules. In that context, our study has particularly scrutinized the Paris area (close to 12 million inhabitants) and the Seine River. Nevertheless, the fingerprints are still incomplete especially concerning the metabolites. Moreover the reliability of data especially at ultratrace level has been highlighted as a main drawback. The aim of this research work was the following: i) achievement of information on occurrence and fate of pharmaceuticals in the Paris area ii) investigation of the relevance of complementary monitoring strategies for a better knowledge of contamination iii) methodological investigation to enable well defined and accurate measurements. All of them are the necessary to sustain a reliable and comprehensive monitoring and decision making process. First, an initial monitoring strategy addressing a set of a hundred relevant pharmaceuticals on representative points of the Paris area (Seine and Marne River) were deployed with the implementation of a “spot samplings-multiresidues methods” strategy. In a second time, it has been completed by a complementary approach based on passive sampler monitoring. Both lead to the selection of a set of pharmaceuticals of main concerns for which reference methods were developed and then uncertainty of measurements were estimated. A specific interest was given to the evaluation of the contribution of sampling uncertainty on the overall measurement uncertainty. Main results, advantages and limits of the implemented strategy as well as lessons will be presented and discussed.

TH077
Development of new materials for passive samplers based on porous organogels
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Keywords: organogels, uptake kinetics, partition coefficient, equilibrium passive sampler
A new type of passive samplers made of porous organogel is under development. An organogel is a semi-solid system in which a liquid is immobilized by a three-dimensional network composed of self-assembled gelator fibers. In the laboratory we have developed an eco-friendly method to introduce and control the microporosity inside the organogel. Sugar crystals are used as water soluble templates to introduce the porosity. Microscopic investigation and 3D microtomography reconstruction of the porous organogel depict the structure of the material. For an organogel prepared with caprylic/capric triglyceride (organic liquid) and 15w% of a gelator (12 hydroxystearic acid), the resulting material revealed interconnected open-pore structures with an effective porosity ranging from 56 to 65 %. Pore sizes ranged from 25 to 500 micron and could be modulated by varying the size of the grains in the template. A large variety of organic liquid can be gelled; consequently the sampler can be adjusted to the polarity of the organic pollutant to be sampled. The porous organogel samplers could either be designed for in situ sampling or for laboratory-based sample treatment. As an example, Bisphenol A sampling will be presented in complex matrices under laboratory conditions. The porous organogel concentrate bisphenol A for further analysis by HPLC-MS detection. Sampling rate was evaluated around 245 $L.j^{-1}$ (unpublished result). As a comparison the value reported with POCIS sampler equals 0.64 $L.j^{-}$ (Li 2011). Pollutants diffuse much more rapidly into the immobilized liquid in the organogel due to the absence of a diffusion barrier. Due to the rapid exchanges with the surrounding media, this new type of materials should be considered as equilibrium passive sampler. Moreover the solubilization ability of the geled liquid allows an important accumulation of the pollutants inside the material for an enhanced sampling. The rapid uptake of this kind of material could help our understanding on both thermodynamic and kinetic control on organic pollutant in the environment at very low concentration. Li H, Helm PA,PatersonG, Metcalfe (2011) Chemosphere 83:271- 280

Measuring and modelling chemical bioavailability in soils (P)

TH078
Determination of leaching behavior of Vanadium from masonry
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Granular recycling and mineral waste materials which may contain e.g. masonry are often used for construction of base layers. Due to the contact to soil or in some cases even groundwater the potential for release of hazardous chemicals has to be determined prior to application. As total contents are a poor measure for the assessment of bioavailability, column percolation tests are used for determination of the leaching potential. The concentration of released components in these eluates can be compared with limit values in the regulations for the intended application. The boundary conditions of the percolation test as flow velocity, contact time between eluent and sample material and the grain size distribution have a possible influence on the results. An important issue for the proper risk assessment is to preferably ensure local chemical equilibrium conditions during this test. Especially for porous material, diffusion from the inner parts to the surface of the particles might be crucial for the overall release. Non-equilibrium conditions may occur, if the transport by diffusion within the material is much slower than the diffusion from the material to the eluent. It is generally expected that larger grain sizes and faster flow velocities favor non-equilibrium conditions. In the here presented study masonry was chosen as example. Eluates of brick material may contain V, Cr, Pb, Mo and As besides alkali and earth alkali elements and especially V is of environmental concern due to its toxicity for aquatic organisms. The influence of the flow velocity (49 and 147 ml/h) and the percentage of the fraction < 4 mm (10% to 90 %) is investigated in a series of column percolation tests. Two brick materials with different porosities were investigated to better understand the interaction between solubility and diffusion as release mechanisms. Furthermore stop-flow experiments are performed using the increase of concentration after the stop as indicator for non–equilibrium conditions. First results show that in general release curves are independent from the amount of fine fractions and the flow velocities. The eluate of the stop-flow experiments show an increase of contaminant concentration after the stop indicating that local equilibrium conditions were not met for both flow velocities and are independent from the percentage of the fraction < 4 mm. These results clearly show that the influence of the grain size and flow velocities is much smaller than expected.

TH079
The relationship between bioaccessibility and chemical form of arsenic associated with iron-oxide in soils
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Many evidence shows that the total concentration of a heavy metal may not adequately represent its realistic toxicity in soil due to its interaction with soil matrix. Such difference mainly results from heavy metal and soil characteristic as well as chemical form of heavy metal in soils. In this study, the relationship between chemical form of arsenic in soil and its bioaccessibility was demonstrated. The five-step sequential extraction method developed by Wenzel et al. and *in vitro* Physiologically-Based Extraction Test (PBET) as a useful tool to determine *in vitro* bioaccessibility of arsenic, was conducted for 24 of artificially arsenic-contaminated soils. Wenzel’s five-step sequential extraction method differentiates operationally five fraction as follows: fraction 1 (non-specifically bound), fraction 2 (specifically bound), fraction 3 (amorphous iron oxide bound), fraction 4 (crystalline iron oxide bound), and fraction 5 (residual). From our results, arsenic was mainly extracted from the amorphous Fe-Al oxide phase, and its contributing to bioaccessibiilty was varied (range from 3 to 58%), suggesting that it maybe depends on chemical bonds type (i.e., adsorption and co-precipitation) between arsenic and amorphous iron oxide. Arsenic adsorbed and co-precipitated amorphous iron oxide, named ferrihydrite, was synthesized, and also extracted using five-step sequential extraction and *in vitro* PBET method. Firstly, the difference characteristic between arsenic adsorbed and co-precipitated ferrihydrite was identified using XPS (X-ray photoelectron spectroscopy) study. Iron and arsenic were present as Fe^{3+} and As^{5+}, and arsenic sorption may occur as replacement of Fe-OH bond to As-O bond in both of synthetic ferrihydrite. However, decreasing the structure density was investigated through arsenic co-precipitated process. From five-step sequential extraction, arsenic adsorbed ferrihydrite was more extracted by weak chemical reagent than arsenic co-precipitated ferrihydrite, indicating arsenics adsorbed ferrihydrite might be more effect on bioaccessibility than arsenic co-precipitated ferrihydrite. *In vitro* PBET test results also showed arsenics adsorbed ferrihydrite (415 mg/kg) was more bioaccessible than arsenic co-precipitated ferrihydrite (67 mg/kg). Our results showed that surface-adsorbed arsenic was more available than co-precipitated arsenic both chemically and biologically.

TH080
Quantifying bioavailable fractions of PAHs in the Swiss Soil Monitoring Network
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Total concentrations of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) in soil may not be related to actual exposures to organisms, because not all pollutant fractions are expected to be equally bioavailable (Reichenberg & Mayer 2006). Due to a lack of agreed standardized methods to assess available organic pollutant concentrations, soil and sediment risk assessment and legal threshold values are still mainly based on total pollutant contents and not on bioavailability. Here, we compared total concentrations of PAHs in samples from the Swiss Soil Monitoring Network (NABO), which is operating since 1985 and has a huge archive with samples from more than hundred observation sites, with bioavailable fractions. As chemical proxies of bioavailability, we used both infinite sink methods with silicon rods (Gouliarmou & Mayer, 2012) and Tenax® (e.g. Cornelissen et al. 2001) as extractants (representing accessibility), and passive sampling with polyoxymethylene (POM; Jonker & Koelmans 2001), representing chemical activity. The results of these three methods will be related to soil parameters such as total organic carbon and black carbon content, and discussed with regard to their potential application in soil pollution risk assessment and legislation. References: Cornelissen, G. et al. Environ. Toxicol. Chem. 2001, 20, 706-711 Gouliarmou, V., Mayer, P. Environ. Sci. Technol. 2012, 46, 10682-10689 Jonker, M.T.O., Koelmans, A.A. Environ. Sci. Technol. 2001, 35, 3742-3748 Reichenberg, F., Mayer, P. Environ. Toxicol. Chem. 2006, 25, 1239-1245

TH081
What’s in our playground? Distribution and bioaccessibility of metals in Canadian urban park soils
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Weathering, atmospheric deposition, industrial activities, automobile exhausts and the use of inorganic pesticides such as wood preservatives and herbicides can potentially introduce metals into urban environments including parks and playgrounds. Incidental soil ingestion is typically the primary exposure pathways especially for young children during recreational activities at these parks and playgrounds. To assess the potential risk associated with exposure to metal contaminants in the urban environment, surface soil samples were collected from parks in various municipalities in Eastern Canada and analyzed for total metals, pH, TOC and metal bioaccessibility. Samples were generally taken from exposed areas within the parks with high potential for human soil contact. The parks were located

in Halifax (10), Saint John (10), Fredericton (10), Ottawa (8), Toronto (15), London (4), Windsor (4), Woodstock (4), Kitchener (4), Guelph (4) and Chatham (4). Arsenic, Cu, Pb, or Zn concentrations in 13 out of the 81 soil samples analyzed exceeded the Canadian Council of Ministers of the Environment (CCME) soil quality guideline for parkland use. Metal distribution reflected the regional natural-occurring differences in metal concentrations and physicochemical properties or anthropogenic sources such as proximity to industrial activities, pesticide treated wooden structures, and heavy traffic corridors. Arsenic concentrations were particularly elevated in Halifax (3.5 – 218 mg/kg) compared to the other cities. The mean metal bioaccessibility values were As (25%), Ba (60%), Cd (84%), Cr (4.2%), Co (24%), Cu (50%), Pb (63%), Ni (14%) and Zn (30%) indicating the relatively high bioavailability of Cd and Pb. There was generally a negative correlation between metal bioaccessibility and TOC while soil pH showed a positive correlation. Based on the total metal concentrations and bioaccessibility data the risk associated with exposure to metals in soils in most of the urban parks studied were deemed fairly low except for As and Pb. For the parks with Pb above the CCME guidelines, bioaccessibility values were also relatively high (up to 99%). Thus Pb in these soils could be potentially bioavailable to humans following exposure through inadvertent ingestion. Ongoing investigations include delineation of the areas with concentrations above the CCME guidelines, identification of the potential sources including metal speciation and sampling in parks in other Canadian cities.

TH082
Pilot Study for Relative Bioavailability Study of PAH in Coal Tar Pitch of Clay Target Fragments
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An *in vivo* bioavailability study is being performed to determine the relative bioavailability of PAHs in clay pigeon target fragments at skeet and trap range sites. The target fragments are composed of PAHs in a site-aged coal tar pitch/limestone matrix. The fragments are mechanically weathered into small particles that are incorporated into the surrounding soil matrix. The bioavailability of PAHs in the soil and target fragment matrix is anticipated to be reduced compared to that seen in animal studies using pure benzo(a)pyrene in solvents added to rodent chow. The Pilot Study includes development of the analytical method and execution of the *in vivo* approach. A high resolution mass spectrometry method has been developed to detect low level PAH metabolites in mouse urine, specifically 3-Hydroxy-benzo(a)pyrene, 9-Hydroxy-benzo(a)pyrene, 7,8-dihydrodiol-benzo(a)pyrene, 3-Hydroxy-benz(a)anthracene, and 3-Hydroxy-chrysene to a reporting limit of 10 pg/mL or lower. The method and method validation procedures will be described. The goals of the *in vivo* pilot study with mice are to: (a) test the methods of preparation and homogeneity of the test articles, which include dried and sieved (250 micron) site soil, site soil extracts, pure BaP in solvents, and pure target fragments mixed into rodent chow, (b) determine the fraction of soil in rodent chow consumed by mice over 14 days, (c) test the urine analysis analytical method with *in vivo* samples, (d) compare PAH metabolite concentrations at two time points (7 days and 14 days) to assist in optimization of the Final Study design, (e) determine the adequacy of urine volume from pooling urine from four mice per sample, and (f) obtain preliminary indications of the relative bioavailability of the three selected PAHs: BaP, benz(a)anthracene, and chrysene. Results to date will be presented.

TH083
Assessment of the impact of organic mater in ndl-pcbs sequestration in industrially contaminated soils : comparison of an in vitro bioaccessibility and an in vivo relative bioavailability assays
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Quantitative health risk assessments are critical issues in management of contaminated soils and involuntary ingestion is one of the exposure pathway studied. Currently, as a margin of safety, 100% of the pollutants from the ingested soil is considered to reach the target organ and to exert toxic effects. However, it is established that only a fraction of the total ingested quantity really enters the bloodstream and reaches the target organs. In recent years, *in vitro* bioaccessibility tests aiming at estimating organic pollutant bioavailability and less expensive and easier to carry out than *in vivo* bioavailability tests, have been developed. Bioaccessibility is the fraction of an ingested compound that could be solubilized in the gastrointestinal tract before being absorbed. Few data are currently available concerning such approaches for organic pollutant contaminated soils. This study was carried out in order to compare bioaccessibility data with relative in vivo bioavailable ones in juvenile swine, a physically close model of young children. It concerned 8 "PCBs contaminated soils" which were collected at five different sites. Soil were freeze-dried and sieved to 500μm and homogenised. Characterization of soils were performed and were notably focused on organic matter (Black carbon amount, carbon organic content). This study involved five juvenile male swine per soil. During 10 days, the piglets were fed one of five doses chosen for one soil to achieve an exposure dose between of 200 and 3 600 ng NDL-PCBs.Kg^{-1} of BW per day in order to assess linearity of dose-response. This soil-fed group was compared to one fed increasing doses of spiked corn oil by Aroclor 1254. After 10 days of oral exposure, NDL-PCBs concentrations in adipose tissue, liver and muscles were determined by GC-MS, after extraction and purification. Linearity assessment were performed on NDL-PCBs levels in tissues using GLM procedure of SAS software. Bioaccessibility of each soil were assessed using the FOREhST procedure. Preliminary results highlight that condensed organic matter is a critical parameter affecting bioavailability or bioaccessibility of PCBs.

TH084
Indicators of metal bioavailability for earthworms in the context of lowly and multi-contaminated soils
L. Beaumelle, Ile de France; M. Hedde, I. Lamy, INRA
Environmental risk assessment of metal contaminated soils needs indicators of metal bioavailability. A number of chemical and biological measurements of metal bioavailability for earthworm exist. Their relevance in situations of in situ contaminated soils must be tested. Especially, the impact of low doses and multiple metal contamination could prevent them from being suitable indicators. In this study, we questioned the use of both chemical and biological measurements to mimic metal bioavailability in the context of soils lowly and multi-contaminated *in situ*. The relationships between metal availability, earthworm metal concentrations and biomarkers responses were investigated in a large panel soils with a range of Cd, Cu, Pb and Zn availability. *Aporrectodea caliginosa* was exposed in the laboratory to 31 soil samples differing in Cd, Pb and Zn total and available ($CaCl_2$ and EDTA-extractable) concentrations, and in soil texture, pH, CEC and organic-C. Energy reserves (glycogen, lipids, proteins), and antioxidant enzymatic activities (catalase (CAT), glutathione-s-transferase (GST)) were recorded in exposed worms. Internal metal concentrations were also measured. Among the different extractions studied, the weakest ($CaCl_2$) appeared as the more suitable to mimic metal availability for earthworms at low doses. While CAT and GST activities were unaffected by metal availability, protein and lipid contents were related to easily extractable metals. However, their high variability and their opposite response to different metals suggest their lack of genericity. Internal metal concentrations did not increase with metal availability, even though earthworms energy reserves were affected. It challenges the suitability of internal concentrations as indicators of metal bioavailability in conditions of low doses and after a short time exposure. Further work is necessary before being able to properly assess metal bioavailability in lowly contaminated soils. In particular, the impact multiple contamination on biological measurements, illustrated in this study, needs to be understood.

TH085
Development of Site-Specific Uptake Factors for Metals
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In ecological risk assessment, bioaccumulation factors (BAFs) are often used to estimate the concentration of constituents in endpoint receptors and prey tissue items. Standard literature-based BAFs for metals uptake from soil are limited to only a handful of metals and have the tendency to over-predict metals concentrations in tissues. Literature-based uptake factors for metals in sediment are even more limited as biota-sediment accumulation factors (BSAFs) are based on the relationship between lipid-normalized tissue and organic carbon-normalized sediment. Accurate prediction of metals uptake is difficult since metals are not lipophilic, persistent organic pollutants that will readily bind to lipid content in an organism. Rather, the accumulation of metals and the resulting tissue concentrations are based on metal uptake and elimination by the organism. Metal uptake is dependent on the concentration and availability of metals in the environment as well as the binding and transport properties into tissue. To identify uptake factors for metals, co-located soil or sediment and tissue (plant and invertebrate) datasets from several sites are examined. Regression plots, using log-transformed data, attempt to estimate a relationship between the soil or sediment and biota tissue datasets. Physical parameters in soil or sediment (e.g., pH, clay content, and organic matter) introduce variability in uptake rates which makes it difficult to establish a regression relationship. Uncertainty regarding the appropriateness of regressions was addressed by rejecting plots that do not possess adequate sample size, goodness of fit, and significant relationships ($p < 0.05$). When regression plots were rejected, uptake rates were based upon the median BAFs calculated using site-specific data. These BAFs assume that accumulation is linear and constant across all soil or sediment concentrations; therefore they do not account for variability in uptake rates across different soil or sediment concentrations, which is why common for many species. This uncertainty is widely documented, which is why regression models are generally recommended over median BAFs for estimating bioaccumulation. However, individual BAFs are considered the next best uptake estimation tool when regressions are deemed inappropriate for use.

TH086
Comparison of batch adsorption and time-dependent sorption parameters of two novel fungicides, penflufen and fluxapyroxad
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Sorption is one of the governing factors that control pesticide mobility in soils. Several studies have shown that pesticide sorption to soil increases with time and can be described as a combination of equilibrium and rate-limited sorption. With an increased proportion of sorbed to the non-equilibrium domain bioavailability of a compound may decrease and, consequently, biodegradation may slow down considerably. Thus, time-dependent sorption could be a reason for bi-phasic degradation behavior of compounds in soil. Taking time-dependent sorption into account may also improve assessment of pesticide transport to groundwater. We studied time-dependent sorption parameters of two fungicides, penflufen and fluxapyroxad and compared them to batch adsorption parameters. Sorption parameters were obtained in batch equilibrium experiments carried out with four soils covering a wide range of soil properties in accordance with protocol OECD 106. The experimental data were well described by Freundlich equations.Organic carbon normalized adsorption coefficients K_{fOC} ranged between 122–311 and 267–623 for penflufen and fluxapyroxad, respectively. Time-dependent sorption parameters were derived from laboratory degradation studies carried out on the same soils according to the proposed "Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments" (Buelke and van Beinum, 2012). At each sampling time the equilibrium phase fraction was determined by a desorption step with water ($CaCl_2$) and the total extractable concentration was analysed by pressurised liquid extraction (PLE) with organic solvent. The fraction sorbed to the non-equilibrium domain was defined as the amount that is not desorbed by water but by organic solvent. Bi-phasic degradation behaviour was observed for both compounds. Degradation was slow with 70–85% of initially applied fungicides recovered by PLE at the end of incubation (day 170). Apparent sorption of both compounds increased considerably over the course of the experiment.Current work aims at implementing the two-site model for kinetic sorption in the PEARLNEQ software and aims at determining reliable modeling input parameters for the pesticide leaching models.

TH087
From lab to field: Integrating field tissue residues, bioaccessibility extractions and invertebrate exposures into a shooting range case study
S.R. Bowman, The Ohio State University / Evolution Ecology and Organismal Biology; J.L. Bryant, The Ohio State University / Department of Entomology; R.P. Lanno, Ohio State University / Department of Evolution Ecology and Organismal Biology
Since metal bioavailability varies with soil modifying characteristics and species-specific uptake and elimination, it is sometimes necessary to use tissue residue measurements and site-specific bioaccessibility measures to evaluate contaminated sites. However, it is not clear how lab bioaccessibility extractions and invertebrate exposures correlate with field-caught mammal and invertebrate tissue levels. Our study is an attempt to combine lab and field data for the same study site to determine how lab data can inform us of field conditions. Our study sites include a private shooting range and an off-site reference area (local park; 8± 1 mg Pb/kg soil) located in central Ohio, USA. Study areas at the shooting range include the main shotfall zone of a trap and skeet range (7248 ± 991 mgPb/kg soil), an area where Pb pellets were excavated (Fall 2009; 1565 ± 36 mg Pb/kg soil), and an area that is similar to the shotfall zone, but does not directly receive lead shot (3521 ± 686 mg Pb/kg soil). We adaptedbioaccessibility assays to small mammal physiology so that we can better estimate exposure to Pb from ingestion of soil and food. Early results suggest that soil bioaccessibility is 5-10% of the total Pb in the shooting range soils. For earthworms (*Eisenia fetida*) exposed to field soils for 30 days, bioaccessible Pb ranged from 9-33% of the total earthworm Pb. We will attempt to correlate small mammal tissue values (shooting range: 121-259 mg Pb/kg liver; off-site reference: 365-1574 mg Pb/kg liver) with these bioaccessibility estimates to gain a broader understanding of how lab extractions inform us of the field condition. Our second objective is to show a comparison of whole body earthworm tissue residues from the field sites (range: 121-1574 mg Pb/kg tissue) and lab-exposed earthworms (range: 3-870 mg Pb/kg tissue) as well as differences between lab and field earthworms in metal rich granule tissue fractions. Future work will include comparisons of bioaccessibility extractions to previously used extractions (weak salt and ammonium nitrate) to understand how our modified method compares to previously used methods. We anticipate that results from our study will provide evidence for the usefulness of bioaccessibility and invertebrate analyses in the site-specific risk assessment of shooting ranges and perhaps more broadly to other Pb contaminated sites.

Mercury Biogeochemistry and Policy (P)

TH088
Mercury photoreactions in Arctic snow
E. Mann, Department of Environmental Science; M. Mallory, Acadia University / Canadian Wildlife Service; S. Ziegler, Memorial University of Newfoundland; N. O'Driscoll, Acadia University
Snow melt in the Arctic is an important source of mercury to water bodies. Prior to the melt period, mercury is lost from snow primarily through photochemical reduction and volatilisation. As such, a quantitative measurement of photochemical reduction is required in order to determine the extent of mercury movement into receiving water bodies with snow melt. To date, studies of mercury photoreduction in snow have been field-based, and as such, subject to many uncontrolled variables (e.g. temperature and irradiation). The goal of this work was to determine the mercury reduction kinetics in snow under controlled (temperature and irradiation) conditions. The analysis system consists of a quartz chamber inside a temperature controlled (-10 °C or 4 °C) Luzchem photoreaction chamber in order to approximate Arctic radiation intensities (1.26 - 5.78 Wm^{-2} for l = 280 to 400 nm). Snow in the quartz chamber is irradiated, while flushing with mercury-free air. Elemental mercury in the air is then quantified using a Tekran model 2537. The elemental mercury produced is quantified continuously (5 min resolution) through time for 24 hours, or until the concentration of mercury liberated falls below the detection limit (0.1 ng m^{-3}). The pseudo-first order reduction rate constant can be quantified through curve fitting to the cumulative elemental mercury data. The mercury photoreduction rate constant was found to range between 0.5 ± 0.07 h^{-1} and 0.1 ± 0.03 h^{-1} for triplicate analyses at each intensity. The data generated from this research can be incorporated into predictive models describing mercury movement in Arctic environments.

TH089
Mercury and methylmercury flux estimation and sediment distribution in an industrialized urban bay
S. Noh, S. Han, GIST / Department of Environmental Science and Engineering; E. Kim, Korea Ocean Research Development Institute / Department of Environmental Science and Engineering
Our objectives of this study were to estimate mercury (Hg) flux to Masan Bay, an industrialized urban bay in Korea, and to examine important factors influencing the Hg distribution and transformation in Masan Bay sediment. As the first attempt to estimate Hg flux for Masan Bay, we found out that stream water discharge was the prime source of Hg, contributing 75% of the total input. Estimating the methyl Hg (MeHg) flux showed that groundwater discharge and sediment diffusion are the major sources, contributing 45% and 23% of the total input, respectively. The large MeHg input through groundwater discharge and sediment diffusion emphasizes the importance of the in-situ sediment production of MeHg. The fraction of MeHg over total Hg (%MeHg) in the bay sediment, ranging from 0.10% to 1.5%, showed a strong negative correlation with the sediment organic matter content (2.8-14% as a loss on ignition). However, when different urban and industrialized estuarine sediments were compared, sediment organic matter either promoted or constrained %MeHg in sediment, suggesting that the role of sediment organic matter for the net Hg methylation varies, relying on the biogeochemical conditions of estuarine sediments. Despite the large variations in Hg and MeHg concentrations among different urban and industrialized estuarine sediments, relatively small variations were determined for the %MeHg, i.e., 0.52% on average. Keywords: methylmercury; methylation; organic matter; flux

TH090
Factors Affecting Mercury Volatilization from Coastal Wetlands
N.J. O\Driscoll, Acadia University / Department of Earth and Enviromental Science; G. McArthur, St Francis Xavier University; J. Canario, University of Lisbon / Instituto Superior Técnico; D. Risk, St. Francis Xavier University; S. Justino, Instituto Superior Técnico; R. Tordon, Environment Canada
This presentation explores research examining the effects of radiation, tidal inundation, and vegetation on in-situ volatilization fluxes of gaseous elemental mercury from coastal wetlands in both Nova Scotia (Bay of Fundy) and Portugal (Tagus Estuary). Mercury fluxes were measured in-situ over diurnal cycles at each site using modifications of a Teflon dynamic flux chamber. In Nova Scotia fluxes ranged from -0.5 to 5 ng m^{-2} hr^{-1} with peak daily fluxes near solar noon and negligible fluxes at night. A rain event allowed for a comparison of the efficiency of radiation-induced flux between pre-rain and post-rain periods, showing that the efficiency was halved in the post-rain period. There was also a noticeable hysteresis effect due to tidal inundation. In addition, the response time of Hg flux to solar radiation decreased from pre-rain (40 minute lag) to post-rain (negligible lab) measurements. Air temperature and tide height were identified to be of secondary importance to Hg fluxes. Overall, our Nova Scotia results indicate that sediment moisture is an important variable determining the extent (and mechanism) of photochemical reduction and flux processes in coastal wetlands. Additional data will be presented comparing recent flux measurements in the Tagus Estuary and the effects of vegetation.

TH091

Estimated annual mass-balance of suspended particulate mercury in a contaminated lagoon environment

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Keywords: mercury, tidal fluxes, suspended particulate matter, lagoon The Isonzo River has been demonstrated to be still a point source of mercury (Hg) in the Gulf of Trieste (northern Adriatic sea) although the Idrija mine definitely stopped his activity in 1996, after 500 years of operation. Due to the erosion of the mining region, of the Idrijca-Isonzo river drainage basin and of the flood plains, fluvial waters remobilize and transport Hg contaminated sediments downstream to the estuarine zone and the marine environment. The present study aims at verifying the role of riverine suspended particulate matter (SPM) associated to tidal fluxes as an important way of Hg dispersion into the Grado coastal lagoon system all over the year. An annual mass-balance of particulate Hg (PHg) in the Grado lagoon was then attempted through several sampling campaigns of the water column at the corresponding tidal inlet. PHg concentrations (avg. 3.11 ± 2.62 μg g^{-1}, d.w.), notwithstanding the ebb or flood tides, were significantly higher than the local sediment background in the Gulf (0.13 μg g^{-1}). The relative affinity of Hg for the particulate phase in surface waters, expressed as log Kd (=[PHg]/[DHg], L kg^{-1}), was also confirmed by higher average values (5.6–6.7) which are in agreement with those previously reported for Isonzo River freshwaters and Gulf of Trieste surface waters. PHg contents, as dry weight but also expressed by volume (ng L^{-1}), showed the highest values in ebb tide conditions thus suggesting to be originated from the erosion of tidal flats and saltmarshes of the Grado lagoon. When compared to daily rainfalls and river discharge trends, high PHg surface concentrations in flood tide conditions are related to rainfalls events occurred within the Isonzo River basin. Results obtained from the tidal inlet can be used to make an indicative assessment of the amount of Hg bound to SPM which is transported in and out of the lagoon basin following the action of tidal fluxes. A simple estimation provides a negative sedimentary budget for the Grado lagoon basin which loses between 0.16 and 1.01 kg of PHg during a tidal semi-cycle. This conclusion is in agreement with evidences of severe morphological deterioration emerged from recent studies on the lagoon environments which testify a current sedimentary loss from the lagoon into the northern Adriatic sea.

TH092

Mercury speciation analysis in marine sediment samples: method validation and occurrence data

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Methylmercury (MeHg) is an acute neurotoxin produced in the aquatic environment from inorganic mercury by sulphate- and iron-reducing bacteria, as well as methanogens. Due to concerns regarding its bioaccumulation in aquatic food chains, much attention has been focused on factors controlling MeHg production. The methylation process mainly occurs in anoxic sediments. Hence, the accurate determination of MeHg in sediments is a crucial tool for understanding the biogeochemical cycle of this potent contaminant. Despite the many improvements achieved in the selectivity and sensitivity provided by most of the analytical techniques commonly used for MeHg analysis, sample preparation remains as the crucial step for Hg speciation. Accordingly, four different sample preparation procedures, namely (1) microwave assisted extraction with 5% (v/v) 2-mercaptoethanol, (2) HNO_3-$CuSO_4$ leaching/CH_2Cl_2 extraction/solvent evaporation, (3) H_2SO_4-KBr-$CuSO_4$ leaching/CH_2Cl_2 extraction/back extraction into $Na_2S_2O_3$ and (4) 25% KOH digestion/CH_2Cl_2 extraction/solvent evaporation were assayed on the basis of the recovery yielded in the extraction of MeHg from CRM IAEA-405 (estuarine sediment). After the extraction, samples were ethylated in aqueous phase, purged out from the matrix and then pre-concentrated onto a Tenax trap. Determination was accomplished by hyphenated GC-pyrolysis-AFS. Procedures 1 and 3 resulted in the fastest methods. However, the MeHg signal obtained by procedure 1 was hampered by the exceptionally high area of inorganic pick, leading to not quantitative recovery (lower than 60%). The validation of the methodology was effectuated according to the ISO-17025 guideline. Thereupon, blanks, selectivity, calibration curve, linearity (0.9995), working range (1-800 pg), recovery, precision, traceability and limits of detection (0.45 pg) and quantification (0.85 pg) were assessed. The estimation of the total uncertainty associated to each measurement result was fundamental tool for sorting the main sources of measurement biases. Preliminary forecast of the uncertainty budgets was used as a strategy to ensure that the determination of MeHg in marine sediment samples could be achieved with demonstrated traceability to a stated system of reference within less than 15 % expanded uncertainty (k=2).

TH093

Mercury in wild gilthead sea bream (Sparus aurata L.) during the first year of life in the Marano and Grado Lagoon (northern Adriatic Sea)

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The gilthead sea bream *Sparus aurata* L. is a warm–temperate marine species, which is common in the Mediterranean Sea and distributed along the east Atlantic coasts from the Canaries and Cape Verde Islands to the English Channel. *S. aurata* is a euryhaline and eurythermal species and a relatively common fish of inshore waters. Postlarval and juvenile stages enter estuaries and lagoon during the early spring and seasonally migrate out of them in autumn. Thus, transitional environments represent a nursery area for this species, which is actively farmed and caught by local fishermen. The Marano and Grado Lagoon experienced an historical mercury (Hg) contamination due to both mining and chlor-alkaly activities. As a consequence this environment suffers from a diffuse sediment contamination, which is actively transferred to the related trophic chain. The aim of this study was to investigate the Hg concentration in the muscular tissue of a selected *S. aurata* cohort during its nursing period in the Lagoon and to compare the results with previous studies. Wild specimens were monthly collected from April 2011 to September 2011 and properly stored at-20°C until the analysis. The total length and body weight were recorded for each specimen and total Hg was determined by direct analysis using the EPA method 7473:2007. A total of 56 samples were analyzed from 497 specimens. Total length (TL) ranged from 3.35 ± 0.2 cmof juveniles to 21.2 cmof the biggest specimen (average TL 12.2 ± 5.6 cm), whereas body weight (W) varied from 0.4 ± 0.1 gto 142 g(average W 37.9 ± 37.9 g) with a calculated length–weight equation of $W = 0.013 \times TL^3$ ($r^2 = 0.988$). Total Hg concentration in muscular tissue ranged from 0.09 to 1.02 μg g^{-1} wet weight (ww) of the smallest and biggest specimens, respectively. Hg concentration at 50th percentiles was 0.4 μg g^{-1} ww and a linear regression was found between Hg concentration and TL ($r^2 = 0.56$, $p \leq 0.05$), W ($r^2 = 0.5$, $p \leq 0.05$) and sampling date in the whole cohort ($r^2 = 0.55$, $p \leq 0.05$). These preliminary data showed a significant level of Hg accumulation, which occurs during the nursing period in the Marano and Grado Lagoon environment. Further investigation are clearly required in order to assess Hg levels in marketable adult specimens and to define the relative percentages of the more toxic Hg form methylmercury.

TH094

Genotoxicity of Mercury in Yellow Perch (Perca flavescens) from a Biological Hotspot in Nova Scotia, Canada

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Anne-Katrin Müller[a]*, Markus Brinkmann[a], Steffen Keiter[a], Karen A. Kidd[b] and Henner Hollert[a] \n *[a]RWTH Aachen University, Institute of Environmental Research, Department of Ecosystem Analysis, Aachen, Germany [b]University of New Brunswick, Canadian Rivers Institute, Saint John, New Brunswick, Canada *E-Mail (corresponding author): anne-katrin.mueller@rwth-aachen.de* Keywords – Methyl mercury, Yellow Perch, genotoxicity Kejimkujik National Park and National Historic Site (KNPNHS) is a remote area in Nova Scotia, Canada without direct anthropogenic pollution. However due to high concentrations of methyl mercury (MeHg) found in yellow perch (*Perca flavescens*) it is known as a biological mercury (Hg) "hotspot". Total Hg concentrations in muscle tissue of yellow perch have increased over the last decade and reached levels between 0.08 to 2.13 μg g^{-1} with MeHg comprising approx. 97 %. A former study in KNPNHS has shown an increased distribution of macrophage aggregates in tissues (liver, kidney, spleen) from perch with high Hg concentrations. Macrophage aggregates are an indicator of cellular damage, which suggests a negative impact of Hg on yellow perch at the cellular level. Therefore the objective of the present study was to determine whether the elevated Hg concentrations cause genotoxicity and ultrastructural alterations on in wild yellow perch. To achieve this, yellow perch were collected in the fall of 2013 from 6 lakes representing a range in Hg concentrations. Tissues from liver and gill were sampled for histopathology and electron microscopy. Micronuclei were counted in blood samples to investigate the genotoxic potential of Hg contamination. These findings will elucidate how Hg adversely affects fish health on the cellular level. Mechanisms of Hg toxicity found in these yellow perch may be also occurring in other wild fish populations inhabiting similarly remote habitats and can help to predict and assess the impact on these populations.

TH095

Effects of mercury and UV radiation on the aquatic macrophyte Elodea nuttallii

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In aquatic ecosystems, anthropogenic contamination with Hg is a topic of great concern. Due to its toxicity, Hg can pose stress to all levels of the ecosystems

including aquatic plants. *Elodea nuttallii* is a rooted submerged macrophyte tolerating a wide range of conditions and has been described as metal bioaccumulator[1]. UV radiation reaching the earth's surface has increased since the 1960, and is predicted to further increase in many regions due to fluxes in the stratospheric ozone layer[2]. Our project aimed to investigate the influence of enhanced UV radiation on the response of *E. nuttallii* to Hg. We exposed *E. nuttallii* to UV (0.55 Wm^{-2} UV_{BE}) for 6 h and to 200 ng/L or 200 μg/L Hg for 24 h or to both. We analysed Hg accumulation, content of the pigments chlorophyll and anthocyanin and oxidative stress. UV radiation decreased Hg uptake in shoots as compared to plants exposed to Hg alone, indicating a possible effect of enhanced UV radiation on Hg fate in natural ecosystems. Pigment content showed a trend to decrease in plants exposed to UV, high Hg concentration, and an additive effect of combined Hg and UV treatment. A similar pattern was observed on lipid peroxidation. Here, Hg alone did not have any effect, but UV slightly decreased lipid peroxidation. Surprisingly, addition of Hg to UV treatment significantly decreased lipid peroxidation in the UV + 200 μg/L Hg treatment. However, possibly the degradation of ROS by oxidative stress responsive enzymes (SOD and peroxidase) was efficient enough to avoid lipid peroxidation. For those enzymes we observed an opposite effect of combined treatment: peroxidase activity was significantly decreased by UV and Hg treatments alone, whereas a combination abolished this effect. SOD activity was enhanced in UV and the 200 μg/L Hg treatments, but all other conditions did not affect SOD. To summarize, the combination of multiple stresses can have a wide range of effects. While for pigment content and lipid peroxidation the effects of Hg and UV were additive, we found the opposite for oxidative stress enzymes. Accumulation of Hg was reduced by UV treatment, indicating that in natural ecosystems enhanced UV radiation could affect Hg distribution in biota. [1] Regier N., et al. (2013) Chemosphere 90, 595-602 [2] Hegglin M. and Shepherd T.G. (2009) Nature Geoscience 2, 687-691

TH096

Discrimination of two geographically distinct populations of sea bass, Dicentrarchus labrax, using stable isotopic signatures of mercury (δ202Hg, Δ201Hg)

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Despite the reduction of mercury (Hg) emissions in Europe in the last decades, Hg emissions are increasing worldwide and concentrations found in some marine predators remain high. This raises questions on mercury's biogeochemical cycle at both local and global scale. In the present work, we investigate the possibility to use mercury isotopic signature in fish as a tool to discriminate different polluted areas and potential pollution sources. Indeed, Hg can exhibit both mass-dependent (MDF) and mass-independent fractionation (MIF). While MDF may occur during biological cycling *inter alia* and could be used to understand bioaccumulation processes, MIF provides a unique fingerprint of specific chemical pathways, such as photochemical transformations. In this context, information provided by Hg isotopes would help to improve environmental management strategies. A preliminary set of four and ten juvenile common sea bass, *Dicentrarchus labrax* were collected from the North Sea and the Aegean Sea respectively. T-Hg was analysed by direct mercury analyser (DMA), speciation by gas chromatography inductively coupled plasma mass spectrometer (GC-ICP-MS) and Hg isotope analysis were performed using cold vapour generation with multicollector ICP-MS. Total Hg concentrations in all tissues were higher in individuals from the North Sea ($Hg_{tot\ muscle}$=1,14 ± 0,48 $mg.kg^{-1}$ dw) than from Greece (0,60 ± 0,06 $mg.kg^{-1}$ dw). Speciation analysis shows that MeHg is the predominant form of Hg in muscle (89% MeHg on average) but not in liver (51% MeHg on average). Isotopic mass dependent values (δ^{202}Hg) values were always higher in muscle than in liver and related to Hg species distribution. For mass independent isotopic signature, sea bass from the Aegean Sea had a systematically higher Δ201Hg value than individuals from the North sea (e.g.: 0,56 ± 0,05‰ and 0,32 ± 0,06‰ respectively in muscle). While mass dependent isotopic signature probably reflects some internal Hg metabolism, mass independent isotopic signature seems definitely site dependent. Such isotopic discrimination might be in agreement with difference in both mercury sources and cycling in the North and Aegean Seas. These preliminary results indicate that Hg isotopes may thus help to discriminate fish from different areas. This promising outcome must be further confirmed by extending the number of individuals and locations to be investigated. \n

TH097

Effect of mercury on periphyton collected in Romanian reservoirs impacted by industrial activities

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Mercury (Hg) is one of the most toxic metals for aquatic environments, particularly under its methylated form, which biomagnifies along the food chain and as such can become harmful for higher organisms. The present study aimed to examine the impacts of Hg towards primary producers in order to get a better understanding on its effects on top consumers. To that end, field experiments were undertaken in the Olt river (Romania), which is known to be impacted by Hg due to releases of a chloralkali plant (Bravo *et al*, Wat. Res, 2013). Periphyton, which are biofilms composed by autotroph and heterotroph microorganisms, were studied as important primary producers in such ecosystems, and as key components of nutrient biogeochemical cycles. In summer 2013, artificial substrata were placed in five different reservoirs along the Olt River representing a gradient of Hg pollution. After three weeks of colonisation, newly formed biofilms were examined for their Hg content as well as for their composition. A translocation experiment was also conducted by exposing periphyton grown in the less impacted reservoir to contaminated reservoirs for 24 hours. Periphytic Hg content was analysed w/wo a washing step with cysteine in order to discriminate between total and intracellular Hg concentrations and measured with an Advanced Hg Analyser. Biotic (algae, fungi and bacteria) and abiotic fractions (particles) were obtained with analyses by epifluorescence microscopy and staining with fluorescent markers. Ambient dissolved Hg concentrations were measured with a Tekan 2600. Physicochemical parameters of the natural waters (pH, temperature, anions, cations, dissolved organic matter concentrations) were also analysed. Dissolved Hg concentrations between the different studied sites varied between 2.40 ± 0.28 pM (control site) and 11.5 ± 4.6 pM (directly impacted site). Periphyton composition was also observed to depend of the studied sites. Indeed, at the control site, artificial substrata were colonized at about 60 %, while at the highest studied Hg concentration, only 28% was occupied. Moreover, algal fraction was prevalent in the less Hg impacted reservoirs with a low cyanobacterial fraction whereas at the most contaminated site, algal fraction was mainly composed of cyanobacteria. Such modification of periphyton composition could ultimately alter ecosystem functioning of the Olt River due to their key roles in nutrient cycle and their level in the food chain.

TH098

Presence of surfactants increase mercury toxicity towards Chlamydomonas reinhardtii

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Ecotoxicogical data obtained in laboratory with model organisms exposed to single contaminant are used to establish water quality criteria. However natural waters contain mixtures of pollutants which effects could differ from those predicted based on single pollutant. The aim of the present study was to examine if the presence of a synthetic surfactant, perfluorooctane sulfonate (PFOS) could affect the toxicity of Hg to green algae. The working hypothesis was that PFOS will increase algal membrane permeability, which will result in an increase of Hg uptake and thus to a higher toxicity of the mixtures compare to the exposure to Hg alone. To that end, the unicellular green alga *Chlamydomonas reinhardtii* was exposed for 48 h to increasing concentrations of HgCl20 (10^{-10} M to 10^{-6} M) and PFOS (10^{-10} M to 4 x 10^{-4} M) alone and in mixture. Several endpoints were measured such as algal growth rate, cell size and granularity as well as pigment content using flow cytometry, coulter counter and fluorescence microscopy. Additional experiments were performed with propidium iodide to assess possible modification of algal membrane permeability. Intracellular Hg concentrations were also measured with an Advanced Mercury Analyser after washing algal cells with cysteine. Algae maintained an optimal growth up to 2 x 10^{-7} M Hg whereas 50 % of growth inhibition was observed at 6 x 10^{-7} M Hg with no further measurable physiological effects. Exposure to PFOS alone induced a decrease of algal growth from 300 μM with 36 % of growth inhibition. Formation of palmelloids, i.e. of colonies of 2, 4 and 8 cells, was also observed with exposure to PFOS and no effect on algal membrane permeability could be detected. Mixtures of Hg with 0.01 μM PFOS and 1 μM PFOS induced similar growth inhibition as exposure to Hg alone. However, exposure to 4 x 10^{-7} M Hg and 6 x 10^{-7} M Hg with 100 μM PFOS led to about 2 times higher toxicity towards algae than exposure to Hg alone, which was accompanied with a 1.4 and 3-fold increase of intracellular Hg concentration. The results demonstrated that algae can be more stressed when exposed to combination of pollutants (here PFOS and Hg) than to pollutant alone and that aquatic organisms may not be fully protected by current water quality criteria without taking into account mixture effects.

TH099

Assessment of inorganic and methyl mercury bioaccumulation in two aquatic primary producers

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Mercury (Hg) is a global concern for water pollution. Both in sediment and water column, fate of mercury may be influenced by the presence of primary producers [1]. Aquatic primary producers are key organisms for ecosystems because they represent a source of oxygen and food at the basis of trophic chains. Assessing Hg bioaccumulation in those organisms is essential for notably predicting its probable impact on higher trophic levels. We studied the accumulation of Hg (inorganic Hg – IHg and methyl Hg - MeHg) in *Elodea nuttallii*, a rooted submerged freshwater macrophyte and in *Chlamydomonas reinhardtii*, a phytoplanktonic species. Both have been shown to play important role in Hg bioaccumulation and biomagnification in freshwater systems food web [2]. We exposed the organisms to IHg or MeHg spiked artificial water in the laboratory and measured accumulated Hg both in kinetics experiments (from 10 min to 8 hours) and wide range of increasing concentrations (10^{-11} to 10^{-7} M Hg). In addition, subcellular localization of accumulated Hg was investigated in *E. nuttallii*, notably inthe cell wall and in the cell sap. Accumulation of Hg from the water is quite rapid in *E. nuttallii*, mainly in the cell wall when exposed to MeHg, or in both cell wall and cell sap when exposed to IHg. This study shows that there is a difference in bioaccumulation kinetics (i.e. assimilation and elimination) between both Hg forms in *E. nuttallii* and we aim at comparing this result to kinetics experiments with *C. reinhardtii*. References: [1] Regier, N. et al. (2012). "Effect of *Elodea nuttallii* Roots on Bacterial Communities and MMHg Proportion in a Hg Polluted Sediment". Plos one 7(9): e45565. [2] Bravo, AG *et al*. (2013). "Extremely elevated methyl mercury levels in water, sediment and organisms in a Romanian reservoir affected by release of mercury from a chlor-alkali plant". Water research (in press).

TH100

Mercury levels in target organs of cetaceans stranded in the Southwest coast of Spain.

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Currently aquatic ecosystems have been exposed to new forms of contamination either by humans or natural processes. In the member states of the European Union chemical quality of surface waters is regulated by the Water Framework Directive (WFD). In this context the decision 2455/2001/CE established a list of 33 substances of priority concern because of their persistence, toxicity and widespread use and detection in rivers, lakes, transitional and coastal waters. Some heavy metals, such as mercury (Hg), are included in this list due to their toxicity, being this element one of the compounds of greatest interest worldwide. Cetaceans are organisms considered useful as sentinels of oceans due to they are sensitive to environmental changes, prone to bioaccumulation and biomagnification, because they are long-lived organisms, and some are top predators. Globally, studies of pollutants in cetaceans stranded have been developed especially in the last ten years. However it is still a field into development, and there are yet few studies taking into consideration a large number of samples and/or different target organs. In this work, levels of mercury in kidney, muscle and liver of stranded striped dolphins (*Stenella coeruleoalba*) in the Southwest coast of Spain have been studied.

TH101

Estimation of mercury bioaccessibility in contaminated agricoltural environment

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The existence of mining activity in Idrija (Slovenia) has been demonstrated to be the primary source of mercury (Hg) in the Gulf of Trieste (northern Adriatic sea) through the Isonzo river inputs. Due to erosion of the mining region, particulate material strongly enriched in Hg was transported downstream and deposited on the riverside soils by the flooding of the Isonzo river. As a consequence the soils of the area are characterized by a diffuse Hg contamination with concentrations reaching up 80 $\mu g\ g^{-1}$, o.d.w. This work was carried out in order to evaluate the application of a bioaccessibility parameter within the calculation of a site-specific risk assessment of these contaminated soils. To evaluate the Hg bioaccessibility, the PBET (Physiologically Based Extraction Test) procedure, considering the risk associated to direct oral intake of contamined soil was applied. Two separate extractions simulating both the stomach and small intestine secretions were sequentially performed. Hg concentration in soil (average $9.41 \pm 6.40\ \mu g\ g^{-1}$) show a clear trend moving downcore, reaching the maximum value of $20.77\ \mu g\ g^{-1}$ in the upper layer. However, the element seems to be accumulated also in the deepest horizon probably due to ingression of contaminated groundwaters. By applying the Bloom et al (2003) selective sequential extraction procedure emerged that Hg is almost entirely in form of cinnabar (HgS) (72.9÷94.1%), whereas the presence of the bioavailable form is negligible. Total bioaccessible Hg, estimated by PBET, ranged from 0.029 to 0.389%, thus confirming the prevalence of HgS form. Moreover, all bioaccessible Hg is present in the stomach phase (acid condition), whereas at the small intestine level (basic condition) no Hg dissolution from the solid phase was observed. In addition to the lab tests, it was evaluated the output obtained with a dedicated software, RiskNet, for the calculation of health risk due to the accidental ingestion of soil contaminated with Hg. The simulation demonstrated that the health risk is close to, or well above, the limit of acceptability. These results confirm that when an evaluation risk analyses is conducted, it is necessary to take into account the whole site-specific geochemistry of the soil.

TH102

Relations between total mercury, methyl-mercury and selenium in five tissues of Sepia officinalis captured in the South Portuguese Coast

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Mercury (Hg) is one of the priority hazardous pollutants in the world, with no role in biochemical processes. Speciation influences Hg mobility in various environmental compartments, being methylmercury (MeHg) the most toxic form. Methyl-mercury is produced in aquatic environment by bacteria and biomagnifies through the food web as result of slower elimination than uptake. Mercury is uptake mainly through diet with MeHg being the form predominantly stored in muscle tissue. Selenium (Se) is an essential trace element known to have an antagonistic action against the toxicity of mercury forms in aquatic organisms. This element seems to have a blocking mechanism in methylation by the precipitation of HgSe or to contribute to MeHg demethylation. The relation Se-Hg has been mainly searched in fish, and fewer studies have been done with invertebrates. The cuttlefish, *Sepia officinalis*, is a nektobenthic species, with high metabolic rates, short life span (around two years), feeding on a large variety of living prey. This work reports the concentrations of total- and methyl-mercury and total Se in digestive gland, branchial hearts, kidney, mantle and gills of *S. officinalis* caught in the south Portuguese coast (Olhão and Vila Real Sto António). The involvement of Se in the mechanism of MeHg demethylation in each tissue was examined. To the best of our knowledge these are the first data on Hg, MeHg and Se in branchial hearts, kidney and gills of cuttlefish. Digestive gland, branchial hearts and kidney presented higher levels of Hg and Se than mantle and gills. Methylmercury was significantly higher in digestive gland, branchial hearts and mantle. The enhanced levels of Hg in digestive gland and branchial heart reinforce the elevated storage capacity of these two tissues. The percentage of MeHg varied from 6.1 % in gills to 92 % in mantle. Linear and positive MeHg-Hg relations were obtained for the five tissues, being the better relation and higher slope observed for mantle, followed by branchial hearts, digestive gland, kidney and gills. The Se:Hg molar ratios showed a surplus of Se in all tissues. Calculations based on the equimolarity of Se:Hg point that 95 to 99% of Se are not linked to Hg (Se free). The negligible quantity of Se associated with Hg suggests that the mechanism of MeHg demethylation was not triggered in none of the tissues, presumably because the threshold for MeHg toxicity was not achieved.

TH103

Hepatic Bioavailability of Total Mercury (THg) in Cichla sp from Tapajós River Region, Brazilian Amazon

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Most of the Hg occurring in the Amazon is of natural origin. However, the intense gold mining activity in the region has increased the levels of this toxic metal, especially in the middle section of the Tapajós River. Specimens of the *Cichla* genus have great economic and ecological significance, as they are the most consumed fish in the Brazilian Amazon region and are at the top of the Amazon food chain. In addition, they are a good indicator of Hg bioaccumulation because the concentrations of Hg in their tissues tend to reflect the accumulation process of successive exposure. A total of 26 adult specimens of *Cichla sp* were obtained by fisheries in the Tapajós River region's contaminated gold mining area. In the laboratory, morphometric data of each fish was taken, for instance total length, total weight and sex. The liver tissue was removed from each specimen, washed with distilled water, packed in polyethylene identified bags and kept at - 20ºC for subsequent total mercury (THg) analysis. Assays were carry out in the total liver and in the cytosol hepatic fraction obtained by ultracentrifugation of the tissue in buffer Tris-HCl 10 mM, pH 7.4. The analyses were performed by flow injection Cold Vapor Atomic Absorption Spectrometry (FIA-CV AAS) with detection (DL) and quantification limits (QL) of 0.527 and 2.585 $\mu g\ Kg^{-1}$, respectively. Individuals of *C. sp* had active gonadal maturation, including spawning and post-spawning individuals. The average length and total weight was 395 ± 51 mm and 840 ± 418 g, respectively. High levels of THg were obtained in the total liver ranging from 1.7 mg Kg^{-1} to 3.8 mg Kg^{-1}. However, only 0.2% of the THg were available to the cytosol hepatic fraction. A significant and positive correlation ($r_s = 0.65$) was found between the concentration of the THg in the total tissue and in the hepatic cytosol fraction. These data are a preliminary study with respect to the bioavailability of total mercury (THg) in different compartments of the hepatic tissue of *C. sp* in order to understand some aspects of the detoxification mechanism of this toxic metal in bioindicator species from the Amazon region. In fact, it was possible to observe differences in the transference of this important toxic metal into the cell and its

linkage in the compartments.

TH104

BIOACCUMULATION AND BIOMAGNIFICATION IN AQUATIC ORGANISMS OF THE ALMADEN MINING DISTRICT

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The Almadén mining district (Spain) is a singular case of natural mercury concentration in the World. The intensive mining activities during the last two thousand years have caused a dispersion and remobilization of mercury within the area. Water bodies in the area are of special concern, specially the living aquatic organisms. This study is focused on the bioaccumulation of Hg levels on organisms of the Valdeazogues River, the main watercourse traversing the Almadén mining district, and to evaluate the Hg trough the trophic chain. In a preliminary sampling campaign, total Hg ranged from 2.21 to 133.70 mg/kg in biofilm, 0.3-20.6 mg/kg in macro invertebrates or 0.6–861.70 mg/kg in crayfish. These results show an enormous bioaccumulation in the specimens collected and a concerned biomagnification in the trophic chain of Almadén freshwaters.

TH105

Identification of present-day and historical sources of mercury in a complex industrial area

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Mercury levels were measured in soils and earthworms on behalf of a long-term surveillance program of the emissions from the industrial area of Scarlino (Southern Tuscany, Central Italy), where a municipal solid waste incinerator and two main chemical plants are currently operating. The study aimed at establishing preliminary soil data to be used as baseline for long-term monitoring of mercury contamination of soil and soil biota and to assess eventual contribution of the industrial emissions. Overall total mercury concentration of superficial soils (0-5 cm), collected from 44 sampling sites randomly selected within a circular area up to 1.5 km from the center of the industrial area, was in average $0.29 \pm 0.27\ \mu g\ g^{-1}$. This value, which remains well below the screening values ($1\ \mu g\ g^{-1}$) for contaminated soils set by the Italian regulatory framework for residential areas, is approximately 4-5 times higher than the European topsoil baseline (FOREGS) for mercury and highlights a general enrichment of the metal in the area. This is mainly attributable to widespread geochemical anomalies characterizing the Southern Tuscany and to the historical mineral processing and smelting activities that have been carried out in the Scarlino area until the mid-90s. The highest mercury concentrations were found in soils from sampling sites in direct proximity of industrial/artisanal activities, where concentrations up to $1.59\ \mu g\ g^{-1}$ were reached. Average mercury concentrations ($0.25 \pm 0.10\ \mu g\ g^{-1}$) from agricultural and grazing land soils were also noticeable, although earthworms from the same sites did not show a significant bioaccumulation of the metal ($0.11 \pm 0.09\ \mu g\ g^{-1}$). The generally elevated concentrations and the high variability (overall coefficient of variation = 93%) of mercury soil data from the Scarlino area are questioning the possibility to isolate the influence of a single source of contamination in an environment with a long industrialization history. This is currently prompting further research supported by techniques for monitoring atmospheric depositions (i.e. biomonitoring, passive sampling) to improve estimation of mercury contamination arising from different possible sources in the Scarlino area.

TH106

Historical variations in the isotopic composition of mercury in sediment cores from northern Adriatic coastal environments

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The results presented in this study involve the analysis of mercury (Hg) isotopes in two sediment cores from the Northern Adriatic. One core was collected in the Gulf of Trieste, an area contaminated by Hg residues produced by the Idrija Hg mine (Slovenia) during 500 years of activity and transported by the Isonzo River to the sea. The second core was collected in the Marano and Grado Lagoon, where Hg from a chlor-alkali plant was discharged into the Aussa-Corno River system flowing into this coastal environment. In this study, we used the high precision stable Hg isotope analysis of environmental samples made possible by continuous flow cold vapour introduction system coupled with MC-ICP-MS. The main aims of this research were to determine the isotope signatures of Hg from the two major sources of contamination, to compare them with the same signatures of the regional sedimentary background and to determine if Hg isotope compositions can be used to distinguish between different products deriving from exploitation and processing of Hg-bearing ore. Analysis of the dated core from the Gulf of Trieste showed that Hg concentration increased sharply at the beginning of 1800s, peaked before the first World War ($23.32\ \mu g\ g^{-1}$ in 1913) and then declined to a constant average of ≈ $8\ \mu g\ g^{-1}$ in recent times (1980s-1990s). δ-values of the $^{199}Hg/^{198}Hg$, $^{200}Hg/^{198}Hg$, $^{201}Hg/^{198}Hg$ and $^{202}Hg/^{198}Hg$ ratios increased upward from the core bottom. Background sediments, where Hg concentration is $0.13\ \mu g\ g^{-1}$, have an average $\delta^{202}Hg$ of -3.93‰ ± 0.92‰ (n=5) which is significantly lower compared to recent contaminated sediments. The isotope signature of Hg deriving from mining activity was not altered by mass-independent fractionation (MIF) related to natural processes. Conversely, it showed mass-dependent fractionation (MDF), due to the transformation processes of Hg-bearing ores (mostly cinnabar but also native Hg in carboniferous schists) to by-products by the retorting processes which caused preferential depletion in the lighter isotopes. The isotope signatures of Hg were quite variable in the contaminated section of sediment cores where $\delta^{202}Hg$ values ranged between -2.95‰ and 0.88‰. The variability in MDF is due to multiple sources, such as unroasted Hg ores and Hg waste calcines which, with different mixture and isotopic fingerprinting, affected the total isotopic composition of sediments.

TH107

Artificial reservoirs downstream the two largest mercury mining areas in the world: source and/or sink of the metal?

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Almadén (Spain) and Idrija (Slovenia) were the largest cinnabar (HgS) mines in the world with an approximate production of 10.5 Million of Hg flasks. During time being in operation and, later, after their closure, a great amount of mercury (Hg) was introduced into the environment. Part of this Hg is still stored in the two mining areas and it is currently supplied to the freshwater aquatic environment due to the erosion of soils, Hg tailings and river banks. Whereas the Valdeazogues River directly drains the mining area, the Isonzo/Soca River receives freshwaters from its tributary, the Idrijca River. Both two drainage basins are characterized by artificial reservoirs along their streams with relatively large dams. They are barriers stopping the normal water flow and they can be efficient sedimentary traps. This study focuses on a preliminary comparison of Hg occurrence in the water column and bottom sediments of the two main artificial reservoirs (Castilseras, in Spain and Solkan, in Slovenia) located along the Valdeazogues and Isonzo/Soca rivers. The aim is to understand the effects of physical and biogeochemical factors on the distribution of Hg species. During field works, both reservoirs showed water column stratification more important in the Castilseras reservoir. The average temperature difference between surface and bottom layers was 12.1°C in Castilseras reservoir, while in the Solkan reservoir the same difference was only 5.6°C, except one point where a karst spring flowing at the bottom of the reservoir determined a significant temperature drop (13.3°C). Most of Hg in the water column is in particulate form predominantly associated with very fine suspended particles. Total dissolved Hg (DHg) concentrations in the water column were similar in both reservoirs (

TH108

Mercury environmental quality standards in the European Union

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Environmental regulatory compliance for Hg pollution has long been assessed in terms of Hg concentrations in the water phase. In such frameworks, Environmental Quality Standards (EQS) are enforced as total Hg concentrations, although methylmercury (MeHg) is the form of highest concern. As a consequence, water quality criteria based on total Hg levels in the water phase may be underprotective against secondary poisoning unless they are adjusted for MeHg levels; a practice that so far is not widespread. Furthermore, monitoring procedures are not necessarily harmonised and even simple filtration before analysis can result in marked differences in Hg levels. To correct for these potential biases, the European Union has introduced, in 2008 and later in 2013, a major shift in Hg monitoring and management by adopting an EQS based on total Hg concentrations in biota as an alternative to the water EQS. The use of a Hg-EQS for biota is scientifically sound

because it automatically takes into account the bioaccumulation and biomagnification processes that are the main causes of concern from Hg pollution. On the other hand, the proposed EQS numerical value (20 $\mu g\ kg^{-1}$ wet weight) risks to create a situation of widespread non compliance in EU water bodies if applied to fish. Directive 2013/39 EU of the European Parliament and of the Council as regards priority substances in the field of water policy states that Member States may classify Hg as an Ubiquitous, Persistent, Bioaccumulative and Toxic substance. Provided that a robust monitoring baseline exists, Member States may present separate classification maps showing that the possible 'not good' status is caused by an UPBT. Hg classification as an UPBT substance must not relax the pressure on Member States to continue targeting important local pollution issues that can cause large differences in neighbouring water bodies. Rather than unduly relaxing a scientifically sound EQS, more state-of-the-art science must be brought in to refine it. We will examine available data for selected European water bodies to show that a biota-based EQS for Hg is scientifically sound and to explore critical issues for consideration and inclusion in further regulatory updates.

Toward sustainability: benchmarks, certification and LCA (P)

TH109

A benchmark for automotive tailpipe (TTW) emissions

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Automobiles are a significant source of air pollution. After power plants, they are generally the second-largest source of greenhouse gases. In Europe, they account for some 60% of nitric oxides (NOx), about 75% of carbon monoxide, and significant amounts of VOCs and particles. The type of fuel or energyused to power an automobile can affect its emissions significantly. Regulators have long known this, and have long encouraged increased use of cleaner fuels. Which raises a critical question – how clean are they, i.e. how do the major fuel types compare on emissions? Existing studies done on a global or regional basis have delivered some answers to this question. However, these existing characterisations are limited in several respects: *Overly aggregated Outdated Based on estimates and models, not empirical testing Cover only gasoline and diesel Cover only some emissions Data from secondary sources* This new study is meant to give a regional (and perhaps global) view on current automotive tailpipe emissions of carbon and local-air-quality pollutants of the following fuels/energies: gasoline, diesel, LPG CNG hybrids fuel cells It is meant to avoid most of all of the limitations of existing studies. This study is based on an unprecedented quantity of test-data – generated by dynamometer testing of commercial automobiles. This makes it more detailed and robust than any other available comparison. The data are current (dating through to September 2013), independent and authoritative. They come from a database compiled by Germany's Federal Motor Transport Authority, the KBA (Kraftfahrtbundesamt). The KBA database contains some 355 thousand individual records of dynamometer emissions-test-data for every passenger vehicle availablein Germany over the past decade. This covers most car models in Europe, and every available fuel type. Results are reported individually, by model and fuel type. KBA data already have been used in two studies that are similar, but more limited in scope, (Heinze & Butnaru, 2012) and (International Energy Agency, 2010). This report also takes the assessment process a step further. Existing studies characterise emissions (weight of pollutant per distance driven), as does this one. The further step – which the existing studies do not attempt – is to assess the relative impacts of these emissions on ecosystems and human health.

TH110

Best environmental practices for industrial parks based on LCA benchmark

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Currently, there is a need to better integrate environmnetal aspects in the construction of new industrial parks to create synergy in the use of resources,and reducing environmental impacts. For this purpose, the use of Life Cycle Assessment (LCA) is relevant because it is one of the main techniques used to assess potential environmental impacts. LCA results can be integrated to benchmarking approaches in view of providing additional meaning to the measurements as on the development of cleaner production practices. The aim of this paper is to present an LCA benchmark case study based on previous investigations done by the Sostenipra research group. The objective is to define better environmentally practices in the construction of industrial parks, focusing in the selection of materials and facilities in streets including sidewalks and underground. We exclude in this presentation the electricity networks, and the public transport. Referent to exploratory review were selected 13 papers referent to LCA studies of facilities systems those were organized in 5 groups (drinking water, rainwater and sewer system, heating distribution, gas distribution e sidewalks). In the case de drinking water can be used the 90 mm diameter pipes with materials as high density polyethylene (HDPE), low density polyethylene (LDPE) and polyvinyl chloride (PVC) In the case of the 200 mm diameter pipes can be used materials as high density polyethylene (HDPE) and PVC polyvinyl chloride (PVC). Referent the Rainwater and sewer system to collect rainwater is recommended use flat rough roofs (i.e 3.8 mm) and as material the flat gravel (FG). To the Heating distribution is important try use the co-utilisation of trenches for different services networks, or service galleries which are multifunctional. On Gas distribution depending the meters to the main grid: >60 m choose propane tank and on Sidewalks exists some materials as asphalt sidewalks that are recommended when the funtional lifetime of the pavements is less than 15 years and concrete sidewalks are recommended when the functional life time is more than 40 years. The facilities identified in the studies can help in the construction of new industrial park with more sustainability focus, however elaborate previous analyses are necessary on the local level to establish what the better practice.

TH111

Environmental footprint of retail logistics as benchmark for sustainable commerce / e-commerce

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In the scope of assessing the environmental footprint of fast moving consumer goods (FMCG), proportionally little attention is given to the environmental implications from retail and logistics. Although traditional retail systems offer limited possibility for differentiation in environmental impacts, e-commerce may lead to more substantial variations in retail impacts. It is the intention of this work to help shape the thinking around sustainable retail assessment and certification. By means of a life cycle model, we have defined an approach to quantify the environmental footprint of traditional "brick & mortar" retail logistics, per single FMCG product item. This model includes the environmental implications of storage, handling, ICT, 'last mile' packaging and all transportation between production and the consumer home. With 'Brick and Mortar' as benchmark, the model offers the possibility for evaluating various forms of e-fulfillment in a modular way. It takes into account the e-tail business model (e.g. pure players, brick& clicks), consumer travel behavior and variations in the 'last mile' logistics (e.g. via collection points and/or different home delivery systems such as parcel or van based). With the availability of such life cycle model, the key parameters that drive the environmental footprint (limited in this study to carbon footprint) can be identified and it offers the opportunity for identifying more sustainable forms of retail/e-tail. A comparison of different purchasing scenarios will be presented.

TH112

Misapplication of generic hazard-classification schemes for versatile, sustainable building materials: Copper as an example

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Inappropriate application of generic hazard-classification schemes for chemicals of concern unnecessarily restricts the use of many versatile, sustainable substances. For example, copper is a versatile building material that is used in many durable forms (i.e., resist decay and have limited water solubility). Copper building materials are sustainable because of their long expectancy of use (e.g., centuries for copper roofs), which drastically decreases the need for replacement materials and associated risk to workers. Moreover, copper is fully recyclable at the end of its use; and the carbon footprint for recycling of copper is minimal. Despite those positive sustainability characteristics and the fact that most copper in building materials is not present as a soluble salt but instead as a metal alloy, copper has recently been misclassified as a chemical of concern (COC). This misclassification results from incorrectly assuming that the fate and bioavailability of copper in soluble salts represent the fate and bioavailability of copper in the wide variety of durable materials used in construction. Although copper released into environmental media has the potential to cause toxicity, hazard does not equate to risk because environmental factors modify potential toxicity. Unlike refined risk assessments, several generic hazard-classification schemes focus on extreme scenarios in which this potential toxicity is highest (e.g., the persistent, bioaccumulative, toxic [PBT] approach). However, the PBT approach is not appropriate for copper.

Human-health risks from exposure to exterior copper building materials and even to copper-alloy antimicrobial "touch" surfaces are negligible; and potential environmental risks to aquatic organisms can be mitigated by simple water-treatment systems, if needed. Copper is a sustainable resource precisely because of its stability and durability, which are positive attributes for its use as a "green building" material. Sufficient scientific evidence demonstrates negligible

risk associated with using copper as a building material. Therefore, the Precautionary Principle does not need to be invoked; and some materials-substitution choices might even lead to undesired consequences. In summary, like some other metals and organics, copper does not need to be listed as a COC, be targeted in material-substitution schemes, and/or be discouraged from use as a building material.

TH113

Carbon payback times for biofuels on a global scale

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The environmental impact of crop-based bioenergy systems is largely dependent on the inclusion or exclusion of land conversion in the impact assessment. First, removal of original vegetation, especially deforestation, sacrifices the carbon sequestration potential in the original vegetation's biomass and soil. Second, the increased land use intensity changes the original soil carbon cycle through farming activities like fertilizer use and irrigation. We use carbon payback time (CPT) as an indicator to assess the life cycle impacts of biofuels. The goal of the present study was twofold. First, we derived spatially-explicit CPTs for global cultivation of six biofuel crops (corn, rapeseed, soybean, sugarcane, sunflower, winter wheat) under three farm management regimes (low input, rain fed; high input, rain fed; and high input, irrigated). CPTs are defined as the number of years required to compensate for the losses in original carbon stocks due to land conversion by the avoided fossil carbon emissions through burning biofuels instead of fossil fuels. Spatially explicit agriculture and forestry models were used to simulate the change in soil and biomass carbon stocks: EPIC for soil organic carbon, G4M for forest biomass, and the classification indices-based model for grassland biomass. Second, we analyze how (1) spatial variability, (2) different crop types, and (3) different management regimes affect the variance in CPTs. We found the largest CPTs in the tropics, where deforestation removes carbon-rich tropical forests. In these regions, CPTs can be larger than 2,500 years. In most of the temperate and boreal regions, CPTs range from 20 to 500 years. We also showed that CPTs are generally smaller under high input farming. Fertilization and irrigation increase the crop yield, thereby increase the amount of biofuel produced per area, and reduce the CPTs. This increase in yield outweighs the increase of GHG emissions caused by higher N and energy inputs during crop cultivation. Statistical analysis showed that 88% of the variance in CPTs can be explained by spatial variability across ecoregions, less than 10% by crop type, and about 1% by management type. We conclude that the environmental impact of crop-based biofuels is highly dependent on the location of the crop cultivation, where the biofuel crop type and management scenario are of less importance.

TH114

Conduction of five environmental declarations in compliance with EN 15804 standard for insulating materials – A Case Study

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Due to recent regulations such as ETS directive (2009/29/EC) in Europe, Grenelle laws including environmental labelling or RT2012 in France, the increasing demand on performance proofs motivates manufacturers to communicate on the global environmental performance of their products. Environmental Product Declarations (EPDs), or Type III declarations as introduced by ISO 14025:2006 standard, have thus become more and more popular among Life Cycle Assessment (LCA) practioners community for building products with the publication of EN 15804:2012 as methodological framework. In France, this standard is completed by a decree aiming at setting additional methodological constraints and indicators for EPDs conduction. In this particular context, IKO ENERTHERM, an insulating material manufacturer, decided to realize five EPDs in compliance with these new frames. The requirements for representativeness and accuracy as well as the methodological complexity of the methods created challenges that needed to be overcome. To comply with the specific French requirements, a cradle-to-grave assessment was carried out according to ISO 14040:2006, ISO 14044:2006, ISO 14025:2006, EN 15804:2012 and a review of PCR published by the German EPD Institute Construction and Environment (IBU) was conducted to extract the best available methodologies. Polyisocyanurate panels are studied with three different facings and four different installation modes (free, gluing, mechanical fixing and edging-stripping) leading to five declarations in total. Data was collected directly on-site for the five products and is representative of the production of 2012. Subsequent to several database and software updates, the 5th version of EIME LCA software was used with its own database of February 2013. LCA shows the highest dependency towards the total environmental impact during the production phase. More specifically, the production of aluminium, used within the facing of the product, is the major contributor to Global Warming Potential and Photochemical Ozone Creation. A ranking of the carbon footprint of the five panels was conducted. A comparison of the five products was conducted highlighting a correlation between the quantity of aluminium in the facings and the resulting environmental results.

TH115

Development of a sustainable process solution for preliminary selection of alternative technologies of water filtration

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Saipem is a EPC/EPCI world leader in the oil and gas contracting services sector, both onshore and offshore. Nowadays clients ask for a more comprehensive process of risk approach to safety and environmental care. The Saipem' AUS (Utilities and Offsite Technologies) department has developed an ALARP (As Low As Reasonably Practicable) approach in preliminary phases of design of utilities. It is a score based multi-criteria approach to decision making that considers health and safety, environmental and social issues, operability, construction, maintenance and costs. At present, environmental issues mainly consider the risks associated to fugitive emissions and the minimization of solid, liquid and gaseous emissions and waste. There is a need for a more quantitative scientifically sounded approach for the evaluation of potential environmental impacts. Thus, Life Cycle assessment has been selected as a suitable method to integrate other decision criteria. The ALARP-LCA procedure specifically aims to improve the preliminary selection of alternative technologies that can achieve the same functional objectives. In the frame of Saipem R&D projects, AUS and ECOAP (Applied Ecology) departments applied LCA to two technologies of sea water filtration for industrial processes: MMF (Multi Media Filtration) and UF (Ultra-Filtration). The study case envisages that filtration utilities are located close to a main harbour in Australia, have a capacity of 400 m3 hour^{-1} and a lifetime of 25 years. They have been compared on the base of reliable design data. Results show that electricity use during operation is by far the most impacting process; that MMF performs better than UF due to the less energy demand; that, on the base of Ecoinvent inventory data, the use of a gas turbine electric generator reduces overall impacts with respects to the Australian electrical grid. Next steps envisage the application of the ALARP-LCA approach to other case studies and the development of an interface tool to expedite the generation of inventory records in LCA software from design data.

TH116

LCA as a supporting tool for building certification

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In the case of construction buliding, sustainability assessment should be done during all life cycle phases. Comparing to other products, the analysis of the environmental impact of a building has to be considered not only during the Cradle to gate and the Disposal phase but especially during the Use phase (i.e. in a Net zero building the emissions are higher in the Cradle to gate phase). Nevertheless building sustainability cannot be assessed as the sum of all materials because their different combination in the envelope could give different energy performances, that is, different environmental impact. Similarly, building materials has to comply with technological and/or aesthetical characteristics in order to reach sustainability criterias required in building certifications (i.e. LEED). In that way LCA has to be intended as a supporting tool for other assessment methods that include also quality indicators. LCA case studies for building are related mainly to material and component omiting aspects as building's location and orientation that contributes to the building energy performance. One of the objective of the study was identify, classify and compare existing european building certifications, focusing on sustainability goals, supporting assessment tools (i.e. LCA and LCC) and other indicators different from environmental ones. In particular it was paid attention to technical quality indicators (i.e. easiness of maintenance, durability, transparency), not measurable with an LCA, that contributes to the achieving of sustainability goals. In a second step a benchmark of technological solutions for the building envelope was selected, focusing on innovation technology in order to identify higher levels of benchmarking for future LCA. Wood and concrete were the two based material selected since having different environmental impact in the production phase. The study was based mainly on grey literature and design methodology was used for benchmarking. Among the findings different sustainability goals and quality indicators have been selected, pointing out the need to include in the assessment, process and technological quality. An indicator based assessment system will be developed by cross-referencing the identified goals with selected indicators. The result of the present work is, indeed, a part of a more extended research regarding the above mentioned criteria and related indicators in order to improve some of the existing sustainable building certifications in terms of new criteria and new levels of benchmarking. Regarding LCA tools, at the moment, they play a subordinated role with respect of other assessment that concerns also qualitative aspects or technical aspects but they have the potential through their methodologies to help sustainability certifications to introduce more precise

quantitive values and, as a consequence, to develope more innovative technologies.

TH117
More than just a phrase: the benchmarking of sustainability performance for industry and policy-makers
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As sustainability embeds in policy and the performance of industry comes under greater scrutiny, the need for sustainability assessment has grown. From lean manufacturing to environmental management systems, from operational efficiency to corporate social responsibility, the need to measure and manage all aspects of sustainability has enhanced. Policy and strategy requires the integration of economic, environmental, and social criteria with the objective of benchmarking efficient performance. Although some methodologies have been proposed, these are still at an early stage of development. The combined Life Cycle Assessment and Data Envelopment Analysis (LCA+DEA) methodology has recently been developed as a valuable framework for sustainability assessment. It can provide sustainability benchmarks that represent efficient practices for a sample of multiple resembling units. The methodology requires thorough data collection for each member of the sample of Decision Making Units (DMUs). The inventory data i.e. material and energy flows combined with socio-economic indicators are used to calculate: the relative efficiency scores and target operating points of inefficient units through DEA, and the environmental profiles of current and target DMUs through LCA. While the methodology´s applicability for benchmarking has already been explored through case studies, this study focuses on two underdeveloped aspects: the establishment of DMU samples via the creation of a consortium, and the potential uses of the sustainability outcomes. The creation of a consortium is a key stage to facilitate data collection. Two types of consortium are considered: consortia of multiple entities from within the same sector (inter-assessment), and consortia of multiple entities within the same company (intra-assessment). A mixture of both types is also considered. The sustainability benchmarks derived from the LCA+DEA methodology could be used by industry and policy-makers for a wide range of purposes, including management (e.g., definition of performance indicators and targets for continuous improvement), decision making (e.g., identification of actions for improvement), policy-making (e.g., definition of reference values) and ancillary uses such as reporting and marketing.

TH118
Life Cycle Sustainability Assessment (LCSA) and Triple Bottom Line (TBL) in Sustainable Product Design
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Sustainability is today one of the priority goals to be achieved in both public policies and business strategies in the international market. A sustainable company should create profit for its shareholders protecting the environment, respecting the workers' rights and improving the ethical values. At the same time, the product development process becomes a critical factor for companies' competitiveness, particularly in eco-innovation. Several methods discussing sustainable business are available in literature, and several tools to support managers in sustainable innovation process. Particularly, two different approaches will be discussed in this paper, the Life Cycle Sustainability Assessment (LCSA) and the Triple Bottom Line (TBL). The LCSA aims to assess all the environmental, economic and social impacts of the product in a life cycle perspective. It is an approach of Life Cycle Assessment (LCA) in a broader context, which includes indicators of the three dimensions of sustainability. The TBL supports the companies to integrate sustainability goals on the agenda business, balancing traditional economic goals with social and environmental concerns, thereby creating a new corporate performance asset. TBL focuses on company's performance in order to assess the relationship between profit, people and planet in company's activities, processes and products. Starting from the emerging debate on sustainable business and sustainable innovation, this research aims to compare similarities and differences between LCSA and TBL through a literature review of scientific papers on the sustainable product design.

TH119
Sustainability Evaluation using life cycle assessment: a proposal for decision makings
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Sustainability is an inherently vague concept whose scientific definition and measurement still lack wide acceptance [1]. However, sustainability is nowadays the goal, in words at least, of the most politicians and decision makers since the publication of the Brutland report in 1987 [2]. The concept of sustainability has gained increasing attention among such problems as global warming, species extinction, overpopulation, poverty, drought, to name but a few, raise questions about the degree of sustainability of our society. The decisions leading to term ought to be based on good science and adequate information. Thus, data are needed about environmental, social, and economical factors known as indicators of sustainability. Some of the questions that must be answered for sustainable projects are: "why unsustainable development occurs", "what is sustainability?", "how can it be measured?", and "which factors affect it? [3]. It is widely accepted that a reliable measure of sustainability should be the result of integrating economic and natural resources accounts. However, this is not readily achievable due to the lack of data and yet unsolved methodological problems. To help to answer sustainability questions, one has to know the meaning of the concept and possess mechanisms to measure it. This paper refines and extends in fundamental ways an existing model for the numerical assessment of sustainability. The proposal uses two different approaches; life cycle assessment (environmental + social) and fuzzy logic to combine a large suite of basic indicators and then computes numerical values of sustainability for a number of composite indexes. At a higher hierarchy it computes the sustainability of an ecological and a social component, and finally, it computes overall sustainability of a process or product. This survey will use a wastewater treatment facility as a case study which is located in the metropolitan area of Mexico City in Mexico.

TH120
The Importance of Uncertainty Analysis in LCA Studies
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Life Cycle Assessment (LCA) is defined as the evaluation of the environmental impact of a product throughout its life cycle, including raw material extraction, manufacturing, and disposal. It is unique in that it uses a life cycle approach to assess environmental impact. LCA is often used in decision-making processes or to inform policy development. Therefore, the accuracy of the results is important to ensure appropriate decisions are made and policies implemented. There are well known limitations to LCA, however, including poor data quality and availability, the use of non-scientific assumptions and decisions, and variations in the computational methodology applied. Because of these limitations, the results from LCA studies have been criticized for their accuracy. It has thus been recognized by many LCA practitioners that uncertainty analysis needs to be incorporated into LCA studies to improve the reliability of the results. This paper reviews the current methods for quantifying and qualifying uncertainty in environmental LCA studies. The classification of uncertainty is discussed, and where each uncertain parameter arises in an LCA is identified. A procedure is given for quantifying uncertainty according to the goal of the study, as quantifying only the classes of uncertainty required to meet the goal will improve the reliability of the results, hence increasing the likeliness of the correct decision being made.

TH121
ILCD Data Network: an IT infrastructure for quality-assured LCI data sharing
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Increasingly, business and government want to asses and communicate the environmental performance of product and organizations. This requires a life cycle approach; considering supply chains, use and end of life along with all associated health, environment, resource and social burdens. Facilitating international networking of data, rather than reliance on any single provider, is essential. This is facilitated by the ILCD Data Network (ILCD DN) that has been launched this earlier year. In its 2013 Communication on "Building the Single Market for Green Products", and in response to commitments in the Communication on "A resource-efficient Europe – Flagship initiative under the Europe 2020 Strategy", the Commission adopted a Recommendation on the use of the Environmental Footprint as methods to measure and communicate the environmental performance of products and of organisations. This ILCD DN consists on the data infrastructure that is critical for further uptake of these and other life cycle based methods for their successful implementation in business and policy decision support. Future scenarios that could beneficially influence LCA data availability are described in the "Global Guidance Principles for Life Cycle Assessment Databases" edited by UNEP. According to UNEP, there is a need for governments, industry associations, and other database providers to take strong action to improve mutual cooperation and to involve many new stakeholders in the LCA community. The UNEP document highlights also the need for an independently managed Data Network using web technologies. All this is fully embraced by the ILCD DN, where several providers are facilitated in sharing data and consequently increasing availability. The data sets in the Network can come from any data developer/owner. The datasets and their publication are independently managed by the respective data developers. In order to guarantee a minimum common quality level and coherence between datasets coming from different sources, there is a need to operate with a minimum set of requirements. The ILCD DN Entry-Level requirements have been established to provide to the user useful information on data quality, guarantee minimum extent of documentation and methodological consistency among datasets. This paper will briefly provide details on the concept behind the ILCD DN, its current status of implementation and future perspectives.

TH122
Practical Life Cycle Inventory Databases: Moving from "what" to "how" in the process to democratize life cycle inventories and applications
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The processes to create goods and services are very variable around the globe, responding to local situations. Ideally Life Cycle Inventory (LCI) databases should include the diversity of these local processes and reflect actual local conditions. Often, primary data needed to develop local datasets exists in the hands of local process engineers, scientists, regulators and the like, rather than in the hands of local Life Cycle Assessment (LCA) professionals. As a result, unit process data collected elsewhere, often modified by the local electric grid data, is used in LCA studies and thus, almost all LCA studies include some proxy data. This situation is observed in developing and OECD countries. Guidance on the content of good LCI databases is available: through the SETAC/UNEP LC Initative Global Guidance Principles for LCA-Databases and the EC ILCD Handbook General Guide for LCA-Detailed Guidance. Such guidance helps describe what should be in a database. The challenge remains to move from primary environmental and process data to high quality unit process data suitable for ingestion by existing databases. At the LCA XIII Conference a group got together to discuss these issues. The outcome was a preliminary outline of what needs to be done, expressed as the goal:*"To develop a generally accepted way to move from primary data collection to LCI data that is transparent, high quality, and inexpensive".* A starting working point is the use of spreadsheet modules based on available examples: 1) Unit process descriptions and primary data collection spreadsheets 2) Spreadsheets that take this primary data and formulate it into LCI data 3) Spreadsheet tools that take this LCI data and format it for ingestions by LCI databases Surrounding these tools, it was recognized there should be an outreach and education program, documents describing how the tools work, educational events for the providers of the primary data as well as LCA professionals globally, and promotional events that encourage the development of the local LCI datasets. Moving forward in a practical way from the "what" to the "how" of LCI data can vastly increase the availability of data, which in turn will increase the accuracy and quality of LCA studies, and democratize the practice of LCA globally. **Key words:** Life Cycle Inventory, Databases, Unit process

TH123
Reporting quantitative uncertainty for life cycle assessment benchmarks in agricultural sector
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According to the latest figures from the Consultative Group on International Agricultural Research, the global food system is responsible for up to one-third of all anthropogenic greenhouse gasses emissions. Recent research works show that there is significant potential for greenhouse gases mitigation in agriculture with the application of measures to enhance the tillage and management activities. To guide the practitioners in this purpose, a tool has been developed to provide information on environmental sustainability (e.g., carbon footprint and water footprint) following the Life Cycle Assessment (LCA) approach based on primary data provided by the grower, with application to different cropping systems and agricultural areas. Within the context of LCA, uncertainty analysis is used to better explain and support LCA conclusions based on the cumulative effects of uncertainty and variability (ISO 14044:2006). Reporting the outcome of the model with a quantitative measurement of the data quality means an added value compared with building deterministic models that obtain results as point estimates. In the presented work, a procedure was included in the model to assess the uncertainty that arise from two types of parameters: activity data and characterization factors. Parameter uncertainty is represented by a lognormal probability distribution using the Pedigree Matrix approach, with which both basic and additional uncertainty can be assigned through variances of the underlying normal distribution. On the one hand, the activity data uncertainty is defined by the user/grower following quality rules depending on the type of data estimation, or by default values according to different types of exchanges. On the other hand, basic and additional uncertainty of the characterization factors is quantified for each LCA vector, following the data quality guidelines for Ecoinvent database v.3. Monte Carlo simulation was discarded as uncertainty propagation method because it is too time-consuming to be implemented in a web-tool, although it is used for the internal validation of the analytical propagation method proposed: Taylor series expansion method. Finally, the impact indicators are depicted with an error bar and they are qualitatively rated by comparison of the resulting uncertainty with the intrinsic variability of the process, which is due to several factors varying with growing season and farm characteristics.

TH124
A methodological approach to improve the reliability and relevance of LCA reviews
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The results of LCA studies on the same product carried out by different authors are often characterized by large results variability, due to the different parameters used as well as the technological systems and impact assessment methods considered. Consequently, the comparability of different LCA studies on the same product or on different products that fulfil the same function is a complex and critical issue. In this regard, LCA literature reviews aim to discuss and summarize the key features emerged from studies as well as highlight how and to what extent the methodological choices affect the results of the assessment. Therefore, it can be a starting point to facilitate a comparison of the results from the LCA studies analysed on a certain product. The aim of this study is to present a methodological approach to harmonise LCA literature studies on different products fulfilling the same function for a reliable and meaningful comparison of their life-cycle environmental impacts. The approach is divided in six steps to rationalize and quicken the efforts needed to carry out the comparison. The steps include: 1) a clear definition of the goal and scope of the review; 2) critical review of the references; 3) identification of significant parameters that have to be harmonised; 4) harmonisation of the parameters; 5) statistical analysis to support the comparison; 6) results discussion. This approach was then applied to the comparative analysis of the published LCAs on tap and bottled water production, focussing on Global Warming Potential results. A statistical analysis with Wilcoxon's test confirmed that the difference between harmonised GWP values of tap and bottled water was significant. The results obtained showed that the harmonised mean GWP results of tap water are lower than the ones of bottled water, even in case of high energy-consuming technologies for drinking treatments. An important strength of the methodological approach is that the harmonisation process enables performing a deep analysis of the existing LCA literature as well as provides useful information for practitioners and decision makers. Moreover, its application to the case study allowed both to supply a description of system variability and to evaluate the importance of key sensitive parameters for tap and bottled water production. The comparative review of LCA studies, with the inclusion of a statistical decision test, can validate and strengthen the final statements of the comparison. Keywords: Comparability, Literature review, Harmonisation, Global Warming Potential

TH125
C-Build e-LICCO database: LCI of building products to find in the ILCD data network
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To perform LCA of buildings, an inventory database of building materials, products and equipment is needed. The quality of the database is of main importance as it directly determines the quality and reliability of building LCA results and conclusions respectively. A state of art of databases available on the French market has shown that INIES (www.inies.fr) and ecoinvent (www.ecoinvent.ch) databases were the main alternatives. Both of them present weaknesses to be used as they are in the French context. Bourgogne district, regional delegation of ADEME in Bourgogne and Cycleco have worked on the development of a database to be used by French building professionals to assess their building in a life cycle perspective: the C-Build e-LICCO database. The work consisted in modelling datasets from the background database ecoinvent. Around 300 building materials, products and equipment, have been created so far, and most of them are parameterised, which result in a total of around 1000 inventories in the C-Build e-LICCO database. Each dataset is composed of elementary flows of ILCD nomenclature and detailed metadata based on ILCD format. 100 datasets have been made available in the ILCD data nextwork and the plan is to integrate them all in the following years. In addition, C-Build e-LICCO is already available in its whole in e-LICCO software. In particular in this web application, datasets are aggregated in constructive systems (wall, floor, roof, etc.) and ratios for fluids and systems work package. This facilitates the use of e-LICCO at the early design phase of a construction process as only three hours are needed to model the entire building life cycle and get LCA results.

TH126
Conversion of datasets in ILCD format – Case study: development of the French national database for textile
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The French government has initiated the development of the environmental footprinting of mass market products. The goal is to provide consumers with the results for three environmental impacts metrics based on Life Cycle Assessment. Two elements are needed: a methodology (product category rules) and a Life Cycle Inventory database. For this, a national LCI database is being developed by the

ADEME (French Environment and Energy Agency). This database, developed in ILCD format, will contain all the data to be used by companies to calculate the environmental impacts of their products and its use will be mandatory in order to ensure data coherence for comparability purpose. The French national database is called IMPACTS database. Due to its international efforts for developing a European LCI database of textile material and processes, Cycleco was selected by the ADEME for providing LCI for textile for the French national database. Cycleco will provide 137 datasets covering a wide variety of textile raw materials and production processes partially aggregated in order to vary the electricity mix according to the country of production. 4 stages are necessary to develop datasets: 1) collect of industrial data and complete it with literature data, 2) modeling and calculation of inventories, 3) conversion of elementary flows in ILCD format, 4) documentation (in ILCD format). The conversion to ILCD format (3rd step), is necessary because the modelling and calculation of inventories is generally done in another format according to the software used (SimaPro format, GaBi format…). Conversion is based on matching elementary flows in original format and elementary flows in ILCD format (mapping of flows). Conversion will be different depending on the choices made during this mapping, which will result in different environmental impacts. Thus, one dataset converted in ILCD format with two converters (two mappings) will have different environmental impacts. Conversion in ILCD format is a key step for the development of textile datasets, and the development of IMPACTS database. This analysis highlights the need to be rigorous while converting data. Any error in mapping can quickly lose the quality of the original dataset. This paper aims at presenting how we managed the conversion in ILCD format for textile datasets: development of the mapping for the conversion from LCA software's output format into ILCD format, study of elementary flows, and differences in nomenclatures.

TH127

Proposal for development of a system for the definition of benchmarks for LCA

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The possibility of defining benchmarks in order to give a sure interpretation of the results of LCA, consistent with international development, this is an issue that must be addressed in a broad and from a general point of view, at the same time, specific for the different identifiable sectors (agri-food, building, packaging, …). Therefore, it is considered appropriate an international organized collaboration among research institutions, governments and industries that will bring together in a single "container" all the rules, the principles and the improvement prospects that will concur in determining the levels of environmental performances, that are going to represent a starting point to define benchmarks. In this way, we propose the idea of preparing an international LCA (web) portal, characterized by geographic area, that could filed under different parameters the results of the LCA applications, which come from different sources (industry, research projects, pilot projects). The classification is going to be done according to the following tree diagram: *geographic area – sector – product – environmental indicator*. By this scheme, it is possible to classify under quantitatively, from which it could be possible to define the average performances, above and below the average, that will respectively represent the value 0 (zero), the positive value (+) and negative (-) one. It could be also possible to define weights related to different indicators, depending on collected data. At this point, in according with an appropriate rating attribution system, we are going to combine weights and benchmark values in order to give a single indicator that could be interpreted in a "maximum-minimum" range of values. Therefore, it would be necessary to introduce a set of positive values (1, 2, 3, …) to define increasing levels of environmental performances, considering type I ecological label (like Ecolabel) too. According with us, this can be a possible step to certificate products or services by Life Cycle Thinking criteria.

TH128

The renewed LCA Resource Directory: a set of self-managed tools for an improved share of Life Cycle Thinking knowledge

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To support EU environmental policies based on Life Cycle approach, the European Commission launched the "European Platform on Life Cycle Assessment (EPLCA)." The platform aims at providing coherent and quality-assured life cycle data, methods, and studies as well as information about developers and suppliers of LCA dedicated software and databases (DB), and service providers. The LCA Resources Directory (RD), has been recently improved, in order to allow the users to register and self-manage their own information, while in the previous version, as regards the contributor's (i.e. developers, suppliers and service providers) section, the entries were managed from the JRC staff only. Significant changes have been also made in terms of content, the new RD includes some templates allowing to upload new types of studies (i.e. Product and Organisation Environmental Footprint, Ecodesign, EPD and related Category Rules/Criteria). Furthermore the old LCA template has been reviewed according to the new structure, and some new fields have been added. New fields have been also added in the tools and DB section, such as the level of compliance or the interoperability with the existing methods/formats (e.g. ILCD entry level, ILCD full compliance, PEF/OEF). After registering, the user is able to insert a set of metadata for the uploaded information, using the templates provided. The models for studies are composed of 54 to 62 fields, depending on the chosen method, while the template for the Category Rules/Criteria accounts 21 fields to be filled. The templates for tool and DB developers contains 21 and 14 fields, respectively. Once the tools and DBs are approved and published in the Resource Directory, the developers are also enabled to validate their suppliers, meaning than when a new user declare to supply the specific tool/DB, the developer is asked to approve it, before the final publication on the public RD. The searching tools have been improved as well, compared to the previous version that merely allowed the full text search. In the new version the users can run an advanced search both within the study and the contributors' section, through the meta-data fields provided during the information upload.

TH129

Comparison of an energy demand and CO2-footprint model for the life cycle assessment of individual housing in two municipalities

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The dependence on non-renewable – and especially fossil – fuels of present energy systems causes manifold adverse impacts on the environment. Given the large energy demands of the built environment, it is obvious that the assessment and the understanding of environmental impacts stemming from housing are essential in order to derive strategies for the abatement of detrimental energy-related effects. Saner et al. (2013) came up with a model for the life cycle assessment of housing and mobility demands of individual households. As an important part of this model, an energy demand model based on simplified energy balances was elaborated for the estimation of space heating, hot water and electricity demand at building level. This model is applied for the municipality of Zernez, a small village in Switzerland. In addition to the evaluation of environmental impacts caused by households, a further goal of this case study is to carry out an in-depth validation of the applied energy demand model. This validation with primary data is facilitated by an extensive dataset which holds information on energy consumption at household level as well as on building characteristics. Moreover, the simulation results of this energy demand model for this village are compared to a previous case study conducted for a mid-sized Swiss municipality. Even though these two municipalities differ from each other significantly in some aspects, the inequality of the distribution of emitted greenhouse gases among households are similar in both cases. According to these results, in both municipalities, approximately 20% of the households are responsible for 50% of the greenhouse gas emissions induced by housing. The results indicate that the applied energy demand model might be a promising basis for further investigations of energy demands in urban areas. The use of well-established and publicly accessible databases facilitates an easy setup and a fast performance of this model. This is an important advantage over laborious, but sophisticated energy demand models.

TH130

Tradeoff identification in comparative life-cycle assessments

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Comparative LCAs and improvement assessment LCAs formulate separate questions that require distinct interpretation approaches. Improvement assessment LCA focuses on the magnitude of impacts for hotspot identification, while the purpose of a comparative LCA is to make salient the most significant trade-offs. However, current standards for interpretation are open and unstructured. This leaves LCA practitioners to apply ad hoc heuristics provided by popular software packages, rather than by application of robust analytic methods. For example, most LCA practitioners leave comparative results as a bar chart or a radar plot. Bar charts may be useful for hotspot identification, but they fail to communicate important tradeoff information. Both, bar charts and radar plots portray mean values alone that do not quantify statistical significance – absolute differences between mean values does not correlate with significance. More importantly, these graphical outputs are mistakenly presented as avoiding normalization and weighting, when in fact, they perform internal normalization and apply equal weighting. Equal weighting represents a narrow value system that does not necessarily concur with those reading the information. To aid in result interpretation at characterization, this study proposes examination of the area between probability distributions as a way to measure tradeoff significance. Standard LCA software packages allow for exploration of uncertainty through Monte Carlo simulation which results in a lognormal distribution for each impact category based on the Pedigree Matrix.

When alternatives perform very similar in a given impact category (yielding a greater overlapping area), the tradeoff becomes less relevant. However, when alternatives have distinct contributions to an impact category (smaller overlapping area), this tradeoff becomes more relevant. Here, choosing one alternative over the other makes a difference for such impact category. This approach can help reduce the list of indicators to the most meaningful to the decision at hand and render a more tractable problem, both cognitively and computationally. Evaluation of tradeoff significance based on the probability distributions of characterized results can help LCA practitioners focus on the most relevant aspects - regardless of weights. Proper tradeoff identification is key in environmental decision making to understand where the compromise lies when selecting one alternative over the other.

TH131

Carbon Performance and Benchmarking of the Printing Industry

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Purpose The carbon assessment and benchmarking of the printing industry is part of the validation procedure for printers to partake in the program for climate neutral printing. The carbon footprint is the basis for accounting of the climate neutral product, which, when offset either by the company or end client receives a label "climate neutral printed matter". As of 2013, 69 print shops in Switzerland participate in the carbon assessment process. Upon implementation of the carbon management system, the print shops are allowed to use the label on their products. To be granted the label, companies are requested to annually reassess the carbon footprint. The program's data collection thus enables a benchmarking process that assesses the print company's performance across time, based on output, specific resources e.g. paper consumption, energy usage or ink/color, and/or in relations to other print companies. **Approach** The carbon assessment is conducted based on ISO 16759, with secondary data from the ecoinvent database v2.1 using the myclimate performance 2.0 software. The data collection is conducted on the basis of consumption per annum of: paper, energy (electricity and heating), print substances, auxiliary material, business travel and commuting. **Innovation and relevance** This is the first carbon assessment in Switzerland based on the data collected from 69 print shops. Due to the group size and comprehensiveness of the data, the carbon assessment is an adequate method to evaluate the environmental performance in the print industry. It can verify efficiency gains in energy and resources consumption achieved through better production processes or technological improvements. The benchmark serves as an industry comparison and can highlight best practices. In addition, it can assist companies to reach targets and lead to industry wide environmental product improvements. **Conclusions and Outlook** This study provides detailed insights into the carbon footprint of the printing industry and supports ecological improvements. It is suggested that the benchmark can provide a foundation for the decision-making process and assist in setting targets for product efficiency. Furthermore, carbon assessment and benchmark can quantify targets through monitoring and verify the achievements.

TH132

Benchmarking of Process Options of Pharmaceutical Reactions

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Based on a mass metric, the pharmaceutical industry has the highest rate of waste generation. In recent years external pressures from society, political agenda and tightening regulatory requirements forced pharmaceutical industry to move towards developing more sustainable processes. According to green chemistry principles this means that a process should produce little or no waste, should not use/produce toxic materials, and the resource use should be optimized. However it is not obvious how to set a benchmark of what is a sustainable process: there is no natural physical benchmark of what is an optimal synthesis unlike, for example, a thermodynamic limit of efficiency in the energy sector.\n\nThe methodologies developed thus far focus on comparative evaluation of alternative chemical routes and incremental improvement and do not allow setting of benchmark targets for new processes. We are interested in metrics methods to support simultaneous development of chemistry and process options using technical, economic and environmental benchmarking on the basis of comprehensive LCA and simplified indicator methods. Such a methodology is required when new process options, such as flow processes applied to pharmaceutical syntheses, open new possibilities for chemical reactions, when comparative assessment of the two alternatives without target setting is insufficient as a decision support methodology.\n\nThe EU SYNFLOW project (www.synflow.eu) has the aim to develop more sustainable chemical processes based on novel catalytic and process technologies. The use of LCA at a very early stage of development is problematic, since there are two many process options and too much uncertainty. Therefore, within SYNFLOW project we developed a three-step evaluation methodology, starting with material proxy indicator, then gate-to-gate process evaluation and finally a comprehensive LCA. In this case we are confident in representing the supply-chain issue dominating chemical routes selection at the early stage of process development, revealing problematic stages of a process using gate-to-gate flow-sheet analysis and, finally, revealing targets for optimisation of the overall process based on LCA and costing analysis. As a comparative benchmark we use the original batch process. However, we also set economic and environmental targets for the new processes based on industrial perspective about the threshold values triggering the decision about adoption of new technology.

TH133

Benchmarking Waste-to-Biogas Plants in Germany: Comparison of Survey Results by Means of Life Cycle Assessment

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In 2012, the amendment of German waste regulation came into action when the cycle economy act (KrWG) replaced the former act on collection and utilization of waste (KrWAbfG). As part of this amendment, separate collection of organic household waste at source is mandatory from 2015 on. Today only about half of German households are connected to a separate collection of organic waste. That is why the collected amount will increase strongly. In addition, many existing plants for organic waste treatment are at the end of their technical life time. Therefore, treatment capacity in Germany needs to be increased to meet the rising amount of waste. Today, most waste is treated by composting, generating a stabilized product usable in agriculture. In the last few years digestion is getting more important. It not only generates products for agriculture (solid and liquid digestate) but also biogas, which can be used as a fuel or to produce energy. Comparison of waste treatment options has already been investigated by LCA. However, existing literature is outdated to a large degree and does not account for the broad variety of technical performance of plants. Thus, a survey among digestion plant operators was conducted to obtain primary data based on reliable information and measured data from plant operators. This survey was conducted based on a questionnaire sent to 122 plant operators. More than one quarter answered the survey and delivered the requested data completely. As an incentive to respond the questionnaire, a benchmarking based on LCA results is included based on mass and energy flow data of plants. Closed plants and plants with inputs like renewable resources or food waste were eliminated from the list. After this elimination 100 plants remained. For benchmarking, the plants were clustered according to main technical characteristics (continuous / batch; mesophilic / thermophilic; high / low solid content). The plants in each cluster were compared to each other and to the average value of the cluster. A comparison between the different technologies was carried out with the mean values of each cluster to identify the most favorable technology. These results can be used for operators in order to optimize performance, but will also be used to derive advice for decision makers in policy and economy.

TH134

Engaging consumers on products environmental impact – Assessment of existing options and recommendations from a company perspective

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Within a sustainability strategy, transparency is the best path to external credibility. Communication through product labeling, whether on product packaging or through other options like websites, is a key communication channel to engage with consumers and drive sustainable product consumption. Natura, a cosmetic company based in Brazil, explored the communication options to engage further their sales representatives, key opinion leader and consumers on sustainability. In order to identify the best options, an analysis of existing labeling initiatives has been performed. Those initiatives were selected to represent the variety of communication and metrics options, as well as best practices. Based on this review of existing solutions, we derived preliminary insights and trends that will be used to derive Natura's product's environmental impact communication towards consumers. Other factors are to be taken into account to frame a communication plan. For example, to select environmental issues to communicate, one should consider the level of awareness of consumers on each issue, the relevance of the issue regarding the products or the business sector and the company's highest sustainability priorities. In regard to communication type, other factors to consider are the transparency level, the comparability with other products within the same or with other companies, the capacity to identify the superiority of one products related to the other, the consumer's awareness again and the ability to express the company's sustsainability goals and progresses. We concluded with the recommendation that a communication of enviornmental impacts at product level shouldn't be based on raw LCA indicators, but rather normalized results or indices. Custom methodologies would need to be developed knowing that standardization is lacking for supporting those solutions as of now. Going even further in the analysis, we recommend to use LCA indicators for identifying environmental issue but to communicate only on indirect environmental issues such a biodegradability, recycled content of packaging, etc linked to the hot spots identified. The main driver for this latter recommendation is the lack of education of consumers, which

need to increased in parallel of the increase in maturity of environmental impact labeling solutions. Thus companies should invest as much in communication than education efforts, which includes different stakeholders and strategy than product labeling.

Developments in Environmental Quality Standards: bridging the gap between science and practical regulatory implementation (P)

TH135

Suggestions for a quality assessment framework for fine, cohesive sediments using nematode-based lines of evidence

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The quality assessment of fine, cohesive sediments is an important issue because they are a major binding phase for many pollutants inside waterbodies. Besides being able to release pollutants to the water phase again, cohesive sediments are directly impacting benthic organisms living between the fine particles. These benthic organisms comprise an abundant and diverse community that fulfils important functions in the aquatic ecosystem, such as regarding the nutrient cycle. Moreover, this community is mainly dominated by meiobenthic organisms (e.g., nematodes, tardigrades, ostracods, gastrotrichs, rotifers) and not by macroinvertebrates. Hence, tools based on nematodes have been recently developed to particularly assess the quality of fine sediments: a) nematode-based Sediment Quality Guidelines (SQG_{Nema}) for estimating the probability of toxic effects based on measured chemical concentrations (Brinke et al., 2013, SETAC Eur. 23rd Annu. Meet., Glasgow, UK, Abstract No. 502), and b) the NemaSPEAR-Index (Nematode SPEcies At Risk) that indicates pollution-induced nematode community alterations (Höss et al., 2011, Environ Int 37: 940-949). The present study demonstrates how both tools can contribute to the chemical (SQG_{Nema}) and ecological (NemaSPEAR) lines of evidence of a cohesive sediment quality assessment framework. A dataset, which includes sites from several river basins across Germany (e.g., Rhine, Danube, Elbe), was used for evaluating the applicability of the tools. Therefore, it was crucial to know which threshold values should be used for the tools in order to classify sediment samples according to their degree of toxic potential and to prioritize sediments that preferentially need management actions in a sound way. Different alternatives for thresholds and weight-of-evidence decisions are presented and compared, including also the use of normalized (e.g., OC) and non-normalized SQGs. The presented nematode-based lines of evidence and the subsequent suggestions for a quality assessment framework will likely facilitate the assessment of fine, cohesive sediments and thus, support decision making processes of regulators. For example, they are aiming at reducing uncertainties that currently exist regarding the role of sediment-bound pollutants for not achieving the good ecological status of (European) waterbodies.

TH136

Derivation of metal sediment quality guidelines for the protection of benthic organisms of the freshwater sediments in Korea

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Sediment quality guidelines of metals for the protection of benthic organisms of freshwater sediments in Korea were derived. A total of 72 sediment samples from contaminated sites were collected by a nation-wide survey. Acute toxicity testings were performed using a standard test species, *Hyalella azteca* (amphipod) and two Korean indigenous species, *Gammarus sobaegensis* (amphipod) and *Chironomus kiiensis* (midge) in parallel with the chemical analysis using ICP-MS and ICP-AES. Using the toxicological and chemical data set, two guideline values (a threshold effect level (TEL), and a probable effect level (PEL)) were successfully developed for Cr, Cu, Ni, and Zn. Prior to derivation of these two guidelines, some toxic data showing chemical concentration less than the background level or geometric mean of nontoxic samples were excluded. The finally derived TELs for Cr, Cu, Ni, Zn were 112, 47.7, 35.9, and 363 mg/kg DW, respectively. The PELs for Cr, Cu, Ni, Zn were 224, 228, 87.5, and 1174 mg/kg DW, respectively. PELs were more than twice higher than TELs. The overall reliabilities (the proportion of toxic samples with above guideline value and nontoxic samples with below guideline value) of PELs were 72 - 77%. In comparison with previously published sediment quality guidelines from Canada, all of our guidelines (the PEL and TEL values) for 4 metals are higher, since the background level of these metals are higher than those of Canada. To evaluate the validity and predictability of our guidelines for applying the sediment quality assessment, an evaluation of the incidence of biological effects within the concentration ranges below and above the guideline values with other chemical and toxicological data sets should be followed.

TH137

Derivation of sediment quality guidelines for polycyclic aromatic hydrocarbons using indigenous benthic organisms in Korea

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Sediment quality guidelines for Polycyclic Aromatic Hydrocarbons (PAHs) were derived using indigenous species in Korea. First of all, the Target Lipid Model (TLM) for PAHs was validated using 10-d sediment toxicity database for indigenous marine amphipod (*Monocorophium uenoi*) against contaminated sediment samples collected from oil spill area. Quantitative Structure and Activity Relationship (QSAR) models with universal slope for narcotic compounds, i.e., TLM, were developed for five different PAHs (2-methyl naphthalane, fluorene, dibenzothiophene, phenanthrene, and pyrene). Using the estimated 10-d LC50 values for individual PAHs including alkylated PAHs, sum of toxic unit were calculated for individual sediment samples based on the equilibrium partition (EqP) model. All of toxic sediment samples showed sum of TU above 1. In addition, QSAR models using another indigenous species such as copepod *Tigriopus japonicus*, andbivalve *Mytilus galloprovincialis*, were also developed. These data were used to estimate the species-specific critical body burden for indigenous species using the TLM. Finally, thirteen species including the above three species were selected and used to estimate HC5 value (0.682 μmol/g octanol) for Korean indigenous species. Final Acute-Chronic Ratio (FACR) was also reviewed and estimated to be 4.85. Using the HC5 value and the FACR, sediment quality guideline for PAHs were derived based the EqP model. In this study, it is shown that toxicokinetc limitation for high molecular PAHs should be considered when PNEC values for individual PAHs were estimated using TLM.

TH138

Assessment criteria for contaminant-related biomarker responses in red mullet (Mullus barbatus) from Spanish Mediterranean waters

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To better assess the ecological objective EO9 included in the ecosystem-based approach (ECAP) of the Mediterranean Action Plan as well as for MSFD purposes, a new monitoring programme of marine pollution will be adopted by Spain in 2015. During the past years, a range of contaminant-related biomarker responses were studied in *Mullus barbatus* from Spanish Mediterranean waters in a coordinated way with chemical measurements. Biomarker responses were yearly measured in 12 specimens (ranging from 12 to 18 cm) for each sex, and they comprised: i) metallothionein content in (MT), antioxidant enzymes, DNA integrity and EROD activity in liver; ii) micronuclei frequency (MN) and Ala-D activity in blood cells; iii) AChE activity in brain; iv) estrogenic activity, PAH metabolites and alkylphenols concentrations in bile. Overall, the results confirmed the occurrence of significant alterations in certain biological responses in fish from marine contaminated areas in comparison to those from reference areas. Results obtained during those surveys prompted to establish the first assessment criteria (AC) for EROD activity, AChE activity and micronuclei frequency in red mullet. Preliminary baseline levels of PAHs bile metabolites, bile estrogenicity in male fish and Ala-D activity in red mullet has been also proposed and all of them presented.

TH139

How effective is the Water Framework Directive in reducing levels of hazardous substances in the Baltic Sea?

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High levels of hazardous substances in the Baltic Sea have been a matter of great concern during the last decades. The Baltic Sea is a semi-enclosed sea surrounded by a large catchment area housing about 85 million people. Hazardous substances are identified as one of four foci areas for the Baltic Sea by the Helsinki Commission (HELCOM). HELCOM has classified the “hazardous substances status” at 144 sites in the Baltic Sea, 40 in the open sea and 104 at coastal sites. The assessment was based on quality-assured monitoring data from 1999-2007 on various chemicals. All open sea areas received a status classification of moderate, poor or bad. Only six of the 104 coastal assessment areas were classified as ”areas not disturbed by hazardous substances”. The most predominant chemicals causing classification status to be poor or bad were PCBs, benz[*a*]anthracene, TBT, mercury, DDE and dioxins. These are chemicals regulated under the EU Water Framework Directive (WFD). The aim of this study is to assess the possibility of the WFD to reduce concentrations of hazardous substances in the Baltic Sea. The WFD is one of very few international directives or conventions concerning the Baltic Sea which actually include measurable objectives in terms of the environmental quality

standards (EQS) for the priority chemicals. The focus of our study is the work carried out by Sweden under the WFD during the ongoing cycle. We assess the chemical status of coastal areas along the Swedish Baltic coast and the management and action plans for the same coastal areas decided by the Swedish Water Authorities. We use this information to analyse what impact the work under the WFD may have on the chemical status of Swedish coastal waters of the Baltic Sea. Our overall aim is to contribute with knowledge to be used for a more cost-efficient WFD and in the long-term for a cleaner Baltic Sea.

TH140

Use of macroinvertebrates and fish to determine priority pollutants concentrations in Walloon Rivers.

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The European Water Framework Directive (WFD) aims to achieve and ensure a good quality status of water in each Member State by 2015. Most of the Environmental Quality Standards (EQSs) are defined for water itself. However standards have also been set in biota for some substances (Directive 2008/105/EC). This is the case of mercury, hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD). Moreover the recent Directive 2013/39/EC adds other substances to that list, including PAHs (benzo-a-pyrene and fluoranthene) and PBDEs. To answer these recommendations we developed methods to measure the concentration of these pollutants in freshwater biota sampled in Walloon Rivers. Three fish species (*Leuciscus cephalus, Abramis brama, Cottus gobio*) and benthic macroinvertebrates were chosen for their interest as sentinel species. Fifty four sites distributed in 3 hydrographic districts and corresponding to the surveillance monitoring sites defined for the WFD were sampled for these organisms. Results obtained for PBDEs, HCB and HCBD are usually in the same range of concentrations in all the considered organisms. However, contamination differences between fish and invertebrates are frequently observed when considering other pollutants such as PAHs and mercury. For example, our results show that benzo-a-pyrene concentration in fish are always These results show that making analysis on different organisms can highlight differences in pollutants accumulation according to the biological group. This allows choosing the most relevant organisms for each pollutant class. Differences in contamination between the studied sites will be discussed.

TH141

Pesticides residues occurrence in Brazilian waters – a review

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Pesticide residues occurrence in freshwater has become a concern in different countries. Brazil, despite occupying the position of world's largest consumer market of pesticides since 2008, does not have an effective monitoring program of pesticide residues in water. In Brazil, the surface water regulation is based on the multiple uses of water, which includes aquatic life protection. In Brazil 380 approximately active ingredients (AI) are registered and used in different crops but less than 10% are regulated. As well as other developing countries, it is common to use criteria defined by developed countries (e.g. North America and Europe), or those from international agencies. Defining water quality criteria is a complex process and needs to be performed by each country, in a scientifically based platform. The very first step is the prioritization of the substances to be regulated and it includes analysis of occurrence data. The aim of this study was to compile the published data on pesticide residues in Brazilian freshwaters. The methodology consisted on searching papers in national and international databases and selecting the data accordingly to a minimal set of laboratory quality criteria previously defined. Data from 15 papers were selected, corresponding to 42 active ingredients investigated in 6,233 analyzed samples from five Brazilian states. These data were retrieved from studies carried out from 1998 to 2010 and published from October 2002 to September 2012. The pesticide residues were found in 14% of the total author’s analyzed samples, corresponding to 31 active ingredients, equivalent to 10% of the total AI registered. Among the active ingredients found in the analyzed waters, 14 are already regulated in Brazil. Fipronil, a non-regulated pesticide, appeared in 67% of the analyzed samples in concentrations up to 26.2 μg/L in the Rio Grande do Sul State. Other active ingredients, such as atrazine, malathion and trifluralin, were quantified by different authors in freshwater above the quality standards established by current environmental regulations. More data is required for the effective identification of priority pesticides in Brazil to start a scientifically based national water quality criteria derivation process.

TH142

The implication of climate change upon future concentrations of phosphate in the rivers of the UK Anglian region

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Summary: This platform presentation summarises the impact climate change could have upon the concentration of phosphate in river in the Anglian region of the UK. This research has highlighted challenges for achieving water framework directive “good” status, due to a likely decrease in flow and increase in phosphate in many of the regions rivers. Finding of the Great River Ouse are presented here. **Abstract**: This research investigates the impact of climate change on the concentration of phosphate in the selected rivers of the Anglian region. Phosphate has been identified by the UK Environment Agency (EA) as one of the most likely reasons for a river to fall short of ‘good’ status under the Water Framework Directive (WFD). The EA has commissioned Atkins to undertake an investigation to determine how phosphate concentrations within rivers in the Anglian region are likely to differ under a changed climate in the 2050s. Source Apportionment GIS (SA-GIS) was used to achieve this. SA-GIS tool was built upon national SIMCAT models that were developed to simulate the distribution of flow at any point within river catchments in England and Wales. The Future Flows Hydrology dataset was used to incorporate climate change into SAGIS. This dataset provides transient projections of daily river flow (and monthly groundwater) levels for an eleven-member ensemble of the Hadley Centre Regional Climate Model (HadRM3). The impact of climate change can be modelled in SA-GIS by perturbing the monthly input values for Q_{mean} (average flow) and Q_{95} (95^{th} percentile flow). As the Future Flows dataset provides transient river flow figures, it is necessary to derive change factors in order to perturb Q_{mean} and Q_{95}. Flows upstream of twenty flow gauge locations within the Anglian region were calibrated to each of the eleven future flow ensembles. Using the Great River Ouse as an example, this investigation demonstrates that the impact of climate change is likely to decrease river flow and increase phosphate concentration. The modelled results indicate that whilst flow could in fact increase (Q16), most of the future flow ensembles have lower flow than the current baseline. This work suggests that, without measures to decrease phosphate emission, climate change may well exacerbate the concentration of phosphate in UK rivers. The work has indicated the need to apply measures to achieve environmental quality standards that are reverent for today and future climates.

TH143

Understanding the chemical speciation of silver in aquatic freshwater systems

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The acute toxicity of silver to aquatic organisms has been extensively tested, and biotic ligand models have been developed for both fish and invertebrates. However when validated there were limitations to their performance. The chronic toxicity of silver has been much less well studied, and the factors modifying bioavailability (i.e. interactions with complexing ligands) are not sufficiently well understood to enable robust models to be developed. Silver is known to form strong complexes with both natural organic matter (e.g. humic and fulvic acids), reduced sulfide ligands (e.g. free sulfide S^{2-}), and also with thiol compounds (e.g cysteine). Attempts to understand the speciation of silver in ecotoxicity tests have revealed significant limitations in our ability to predict silver speciation under different water chemistry conditions. The relative importance of the different strong silver-complexing ligand types, as well as uncertainties regarding their concentrations in natural systems, means that a meaningful interpretation of the existing chronic ecotoxicity data for silver in terms of biotic ligand model approaches is not currently possible. The reasons for these uncertainties, and the approaches which may be taken to address them, will be outlined. The key aims of this study are to determine silver speciation in a range of aqueous systems, from simple well defined systems with individual ligands to complex systems with multiple ligands. This information will be used to determine how well WHAM VII predicts Ag complexation in receiving waters in relation to variable DOM-sulfide and free sulfide concentrations, and to calibrate and validate WHAM VII for the prediction of silver speciation in natural systems if necessary. This may require that sulfide ligands and their silver complexes are explicitly included in model calculations. Whilst it is clear that dissolved silver can be highly toxic to some aquatic organisms, a better understanding of the conditions which are likely to result in toxicity to organisms and ecosystems would lead to considerable advances in understanding the potential risks from silver in surface waters.

TH144

Parameterisation of the Nickel Biotic Ligand Model for an Australian Species

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A bioavailability-based approach is now being implemented across the EU for metals for which biotic ligand models are available. The recent EU Proposal for Environmental Quality Standards to apply EU-wide includes bioavailability-based EQS for nickel. Site-specific Water Quality Guidelines avoid the obstacles associated with Guidelines that are based on single-value, reasonable worst-case approaches, while maintaining a standard level of ecological protection (e.g.,

protection of 95% of aquatic species). Interest from regulatory authorities in other regions has prompted an assessment of the applicability of the nickel chronic biotic ligand model (BLM) to an Australian species. The research aims to validate a currently existing Ni bioavailability models for an Australian species in field collected water samples. Five natural waters have been selected to represent the range of different water chemistry characteristics found across Australia. A standard Australian test species has been tested for its sensitivity to nickel in each of these waters, exhibiting a range of sensitivities depending on the water chemistry. Analysis of the predicted nickel speciation in the test waters, and competitive interactions between different chemical species at the "biotic ligand" allows the intrinsic sensitivity of the test species to be defined in terms of the critical level of nickel accumulation at the biotic ligand. A comparison between the observed and model predicted results is presented, with recommendations regarding the applicability of the nickel BLM to Australian species for water quality standard derivation. Validation of Ni bioavailability models for Australian species in Australian waters is an important aspect of demonstrating the applicability of the previously developed BLMs to other species within the relevant trophic level. Second, the validated model and the integrated dataset will provide the means to generate site-specific Water Quality Guidelines for Australian waters. The results of ecotoxicity tests performed in a number of different Australian waters will be presented, along with predictions of the fitted bioavailability model for the species. An appraisal of the ability of the existing nickel BLM to describe the toxicity of nickel to an Australian species, assuming that only the intrinsic sensitivity of the organisms needs to be specifically fitted, will also be presented.

TH145

Water quality standards for imidacloprid – new values to protect aquatic life

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Imidacloprid is among the pesticides that most frequently exceed current water quality standards in the Netherlands. In 2013, the European Commission has adopted a proposal to restrict the use of imidacloprid (neonicotinoid) in response to a scientific report of the European Food Safety Authority (EFSA) on bee health. Exception to the restrictions are applications in greenhouses and full-crop applications that take place after flowering. This means that applications with potential emissions to surface water will not directly be affected by the restrictions. The present water quality standard for chronic exposure (AA-EQS) is 67 ng/L, the standard for peak exposure (MAC-EQS) is 200 ng/L. These values were derived in 2008. During the past years, a large number of new aquatic ecotoxicity studies have been published, apparently triggered by the ongoing societal debate on neonicotinoids and their potential impact on bee health. The new data include chronic studies on aquatic insects, for which in 2008 only few acute studies were available. For mayflies, that are particularly sensitive, long-term exposure results in effects at concentrations that are lower than the present AA-EQS. This indicates that the current AA-EQS is not protective for long-term exposure to imidacloprid. RIVM reviewed the available literature, and derived new standards using Species Sensitivity Distributions. The results of nine micro- and mesocosm studies were included in this evaluation. Depending on the method used, the new AA-EQS will be a factor of 3 to 8 lower than the current standard, while the previously set MAC-EQS is confirmed by the new data. Based on the new standards it is expected that imidacloprid remains a problem in the Netherlands, unless measures are taken to prevent further emissions to surface water.

TH146

A systematic comparison of the user-friendly tools used to estimate the bioavailability of Cu, Ni and Zn in the aquatic environment of Europe

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The development over recent years of acute and chronic biotic ligand models (BLMs) for metals such as Cu, Zn and Ni has demonstrated that the bioavailability (and associated hazard) of metals in aquatic environments is dependent on water physicochemistry. Water physicochemistry is described by a range of variables, including (but not restricted to) pH, major ions, major anions, alkalinity, temperature and dissolved organic carbon (DOC). As such, Environmental Quality Standards (EQS) based on bioavailability offer the most appropriate means of regulating the hazards of metals in the aquatic environment. BLMs require an extensive suite of input parameters and, despite offering the most realistic approximation of metal bioavailability under different physicochemical conditions, are likely to be too complex for routine regulatory implementation (i.e. under the Water Framework Directive). In response to this situation, several “user friendly” bioavailability tools based on BLM concepts have been developed for regulatory implementation. These tools calculate site-specific metal bioavailability using fewer inputs than BLMs, as well as offering additional functionality (i.e. tools for undertaking compliance assessment). Any loss in the precision of bioavailability estimates relative to BLMs is considered to be balanced against the costs savings associated with greater sample throughput and reduced monitoring burden relative to the full BLM. Several user friendly bioavailability tools have been developed: bio-met bioavailability tool, PNECPro and M-BAT. It is important that all tools meet a minimum standard of performance in terms of precision, accuracy and applicability domain relative to BLMs, in order that regulatory decisions based on the tools are scientifically defensible. This poster describes a systematic and objective evaluation of the performance of the three previously mentioned tools in terms of 1. Scientific rationale and underpinning data 2. Performance relative to the BLM, and 3. Utility of tool within regulatory and industry decision making frameworks.

TH148

Wrong conclusions from correct results in metal toxicity testing

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Trace element speciation is a key factor in controlling metal bioavailability and ecotoxicity to aquatic organisms. In this respect, standardized ecotoxicological protocols and test media can ensure that element speciation is comparable in experiments performed across laboratories and that available data can be pooled during risk assessment procedures. Trace elements are typically introduced in laboratory culture media in soluble forms (e.g., chloride salts), but the characteristics of many culture media can promote the formation of insoluble species (e.g., carbonates, oxyhydroxides, phosphates) in the case of some trace elements. In such situation, effective exposure concentrations for test organisms may rapidly and markedly decrease during testing, potentially leading to underestimation of toxicity and errors in risk assessment. In this contribution we use a chemical speciation model to estimate the likelihood of the formation of insoluble chemical species for different elements and, for selected examples, compare model predictions with experimental results. Possible ways to deal with the formation of insoluble species during ecotoxicological tests are discussed.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (P)

TH149

Cyanobacteria as natural sources of retinoids in the environment

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Retinoids (known as dietary hormones) derive from dietary sources of vitamin A or its precursors. They play a role as important signaling molecules which control vital cell processes like morphogenesis, development, reproduction or apoptosis. It was documented that these receptor-mediated processes can be affected by some environmental pollutants. Our study highlights the ability of some species of cyanobacteria to produce retinoids naturally and excrete them directly into the environment. We present results obtained from in vitro screening of a wide range of laboratory-cultured species representing several orders of cyanobacteria and algae. The retinoid activity was determined in murine embryonic carcinoma cell line P19 transfected with luciferase reporter pRAREβ2-TK-luc, which contains reporter luciferase gene under the control of retinoic acid-responsive element. The total content of retinoids in samples was expressed as all-trans-retinoic acid equivalents (REQ). The results document differences in intracellular content of retinoids and extracellular production of retinoids among assessed cyanobacterial species. In case of algae none of the tested exudates showed retinoid activity which would exceed limit of detection of 30 ng REQ/L. The most effective cyanobacterial exudates contained thousands ng REQ/L. We also monitored the influence of abiotic factors, specifically light intensity, on the production of retinoids. To sum up, our results confirm the production of retinoids to the environment even by common widespread species of cyanobacteria. High concentrations of retinoids were repeatedly detected for Cylindrospermopsis and Microcystis species. The work was supported by the Czech Science Foundation grant No. GACR P503/12/0553.

TH150

Safety evaluation of three potential biofuels with respect to estrogenic activity

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Biofuels for the transport sector are considered renewable alternatives with the benefit of reducing the dependence on fossil fuels and a potential to decrease greenhouse gas (GHG) emissions. Consequently, biofuel production increased dramatically in recent years. Growing production capacities and the associated rise in biofuel consumption also increased the risk of a release into the environment or a potential direct contact with human beings. However, no publications regarding estrogenic activity or potential endocrine disrupting potencies of biofuels are available. In this study we investigated the cytotoxicity on US-O2 cells and the estrogenic activity in the ERα CALUX® of selected substances derived from biomass and with promising properties for the use in combustion engines (ethyl levulinate, 2-MTHF, 2-MF). The results were compared to the estrogenic activity of

water accommodated fractions of fossil fuels (diesel and gasoline). In the cytotoxicity test 2-MF showed the highest but marginal toxic potential with an EC_{50}-value of 833 mg/L. For the fossil fuels a reduction of the cell viability by more than 15% was not found at the concentrations tested. The ERα CALUX assay revealed no estrogenic activity for any of the potential biofuels at concentrations below the EC_{20}-value. Likewise, estrogenic activity was not found for the fossil fuels. In conclusion, the results reveal no estrogenic activity in the ERα CALUX. However, the investigated potential biofuels might not be representative for biofuels produced in the future. Therefore, further investigations of potential biofuels or biofuel components in test methods for detecting potential endocrine disrupting activity should be conducted to exclude an impact on the environment or human health. **Acknowledgement:** This work was performed as part of the Cluster of Excellence "Tailor-Made Fuels from Biomass", which is funded by the Excellence Initiative by the German federal and state governments to promote science and research at German universities.

TH151

Endocrine disrupting activities profiling of monitored French river sites based on in vitro bio-analytical tools

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Regarding thousands of chemicals occurring in aquatic systems at trace levels and as mixtures, current regulatory tools -in the frame of the water framework directive (WFD)- give an incomplete scope of environmental complexity. In this context, development and evaluation of new strategies allowing holistic contamination characterization and identification of environmental pollutants contributing to deleterious effect (e.g. endocrine disruption) on exposed organisms is relevant. To tackle this challenge, the use of *in vitro* bio-analytical tools is a promising approach since they allow specific, sensitive and quantitative detection of all compounds presenting similar mechanism of action (e.g. binding to nuclear receptors) within environmental mixtures. Hence, monitoring active pollutants by using a panel of complementary bioassays should increase the detection capacity of contaminants and allow a better hazard assessment through biological activity-based profiling. In the frame of a large study on surface waters monitoring in France in 2012 (Botta et al. accompanying communication), we applied a battery of receptor-based *in vitro* assays (estrogen (ER), androgen (AR), glucorticoid (GR),pregnane (PXR), and aryl hydrocarbon (AhR) receptors) for establishing endocrine disrupting profiles of 20 French rivers sites subject to various anthropogenic pressures (i.e. urban, agricultural, industrial, mixed, reference). This was performed on both sediment and surface water (both grab and POCIS) extracts. Estrogenic (1-10 ng-E2eq/g d.w range), PXR-like (1-10 μg-SReq/g d.w range) and dioxin-like (1-100 ngTCDD-eq/g d.w range) activities were detected in both sediment and surface water while anti-androgenic activity (10-100 μg-DHTeq/g d.w range) was only detected in sediment and glucorticoidic activity (100 μg-DEXeq/g d.wrange) was only detected in one surface water.Overall, the levels of detected activity differed according to sites and to compartments. Altogether, the use of *in vitro* bio-analytical tools combined to multi-compartment approach offers a cost-effective integrated quality assessment of the chemical status and permits to classify and identify hotspot polluted sites.They constitute a relevant complementary approach to current regulatory chemicals analyses for routine monitoring of chemical quality status.

TH152

Comparative study of in vitro and in vivo methods for the detection of endocrine disruption in aquatic invertebrates

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Aquatic ecosystems constitute the chemicals’ final destination. One of the chemicals’ families targeted by the EU Water Framework Directive is pesticide due to their endocrine disruption (ED) potential. Nowadays, ED effects of chemicals are well documented in vertebrates and the Organization for Economic Co-operation and Development (OECD) recommends the use of human receptor (estrogen or androgen) assays for the screening of ED chemicals. Few data are available on the ED effects on invertebrates whereas they represent 95% of the wild fauna. In order to determine if the vertebrate data set is relevant in invertebrates, we have undertaken to compare the sensibility of (1) a crustacean *in vivo* test *i.e.* lethal toxicity test in the copepod *Eurytemora affinis* and (2) standard *in vitro* tests *i.e.* recombinant yeast estrogen and androgen receptor agonist and antagonist screening assays (YES/YAS) using model compounds *i.e.* pyriproxyfen (PXF) and chlordecone (CLD), alone and in a binary mixture (PXF/CLD). Five concentrations of each pesticide and binary mixture have been tested: 10 to 400 $\mu g.L^{-1}$ for PXF, 10 to 200 $\mu g.L^{-1}$ for CLD and 2.5 $\mu g.L^{-1}$ / 3.75 $\mu g.L^{-1}$ to 25 $\mu g.L^{-1}$ / 37.5 $\mu g.L^{-1}$ for PXF/CLD. The biotest - using the brackish sentinel copepod *E. affinis* - was conducted according to the ISO standard. Briefly, it consists of a semi-static exposure of three replicates of ten copepods to a range of concentrations of the investigated chemical in water (15 PSU) for 96h. Dead copepods were counted and removed every 24h. For each pesticide, the NOEC, LOEC and LC_{50} 48h were calculated. Moreover, the compound ability to mimic estrogens and androgens were respectively evaluated by YES and YAS assays using the same range of compound concentrations. The CLD NOEC and LOEC values were lower than the positive ED concentration recorded by the YES/YAS assays. Furthermore, no ED was highlighted for PXF and PXF/CLD with YES and YAS while effects on the copepod have been recorded after *in vivo* exposure. Even if the *in vitro* assays are interesting screening tools, the present study shows that no direct extrapolation can be made from *in vitro* tests to estimate the pesticide toxicity on aquatic invertebrate such as *E. affinis*. To further investigate the ED potential of the model compounds on *E. affinis*, reproductive performances (*i.e.* number of eggs per female or sex ratio) will be evaluated.

TH153

Characterization and comparison of transcriptional activities of the retinoid X receptors by various organotin compounds in three prosobranch gastropods

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The organotin compounds have a high affinity for retinoid X receptor (RXR), which is a transcriptional factor activated by retinoids that induce imposex in gastropods. The molecular mechanisms underlying the regulation of RXR, however, have not been clarified in gastropods yet. In this study, we isolated new two cDNAs encoding *B. japonica* RXR isoforms, and characterized the RXR isoforms of three different prosobranch gastropods, *Thais clavigera* (*TcRXR-1,* and -2), *Nucella lapillus* (*NlRXRa,* and *b*) and *Babylonia japonica* (*BjRXR-1,* and -2). The deduced amino acid sequences of these cDNAs showed that these RXRs were very similar each other. The transcriptional activities of RXR isoforms of each species were activated by 9-*cis* retinoic acid (9cRA) using COS-1 cell.Transcriptional activities of TcRXR-2, BjRXR-2 and NlRXRb were lower than those of TcRXR-1, BjRXR-1 and NlRXRa with retinoid X receptor responsible element (RXRE) reporter vector, respectively. However, transcriptional activity of TcRXR-2 was significantly higher than that of TcRXR-1 when thyroid hormone responsible element (TREpal) -reporter vector was used. The transcriptional activities of BjRXR-2 and NlRXRb using TREpal-reporter vector also denoted higher activities than BjRXR-1 and NlRXRa, respectively. We also demonstrated the transcriptional activations of TcRXRs, BjRXRs and NlRXRs in COS-1 cells by mammalian RXR specific agonist, PA024, and 16 organotin compounds. Transcriptional activities of these RXR proteins were activated by PA024 using each three species’ RXRs. We also found a ligand dependent-transactivation using RXRE-reporter vector by 5 organotins: tributyltin (TBT), tetrabutyltin (TeBT), tripropyltin (TPrT), tricyclohexyltin (TCHT) and triphenyltin (TPhT). Interestingly, those transcriptional activities were suppressed by the addition of mammalian RXR specific antagonist, HX531. These TBT, TPhT, TPrT and TCHT are reported to induce/promote the development of imposex in *T. clavigera* after a month of injections of each of the compounds. These results suggest that the development of imposex is induced by certain organotins through RXR cascade in prosobranch gastropods.

TH154

Reproduction test with a mollusc species under consideration of two different sexual endocrine mode of actions

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To assess the effects of endocrine disrupting (ED) substances on aquatic invertebrates, a couple of OECD test guidelines have been and are being validated. A partial life cycle toxicity test with the mudsnail species *Potamorpyrgus antipodarum* is in the process of validation. This species is considered to be rather sensitive with regard to endocrine disrupting compounds. In this study two tests using *P. antipodarum* were performed. As reference study, a test with the potent estrogen receptor agonist ethinylestradiol was conducted. This test was used to check sensitivity of the snail batch. Results from this test were compared with data from the literature. In a second study, the non steroidal aromatase inhibitor fadrozole was applied. In both studies, the reproductive success was examined by counting the number of embryos per adult animal at test termination. While the estrogen induced an increased reproductive output, the aromatase inhibitor clearly reduced the number of embryos. The results allowed conclusions on the sensitivity of the test species with regard to different sexual endocrine mode of actions, represented by both receptor and enzyme-interaction. Within this study, also the sensitivity of the exemplaric mollusc species was compared with other organisms

which had been exposed to the same substances.

TH155
Exploring the value of gene expression biomarkers for existing test guidelines to detect endocrine disruption in fish
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To assess the effects of endocrine disrupting (ED) substances on fish, a couple of OECD test guidelines have been and are being validated. The analysis of biomarkers within these test is usually limited to the measurement of proteins like vitellogenin or spiggin in blood plasma or liver. The analysis of further physiological parameters like steroid hormones is not intended, but molecular parameters may represent a useful addition for interpreting observed apical effects, as they exhibit a very short and sensitive response to exposure. Moreover, recent progress in genomics research will significantly improve the interpretation of molecular mechanisms in terms of population relevance. In this study, a Fish Sexual Development Test (FSDT) was conducted and extended by the measurement of expression profiles of selected genes. The aim was to test whether the inclusion of molecular endpoints may improve the explanatory power of the existing FSDT. Hence, an FSDT was conducted with zebrafish in compliance with the OECD TG 234 and following GLP principles. The nonsteroidal aromatase inhibitor fadrozole hydrochloride was chosen as test substance and the exposure took place in a flow-through system at 10, 32 and 100 µg/L. LC-MS method was used to confirm the exposure concentrations. Real-time quantitative PCR (qPCR) was applied for gene expression analysis in zebrafish embryos (at 48 hpf) and eleutheroembryos (96 hpf) as well as in 28 dpf and 63 dpf zebrafish. Measured genes were selected based on previous in-house microarray study results for 48 hpf zebrafish embryos and according to literature data for adult zebrafish. At the time of abstract submission, preliminary results on hatch, early-life survival and gene expression indicated effects on survival at 32 and 100 µg/L and on the expression of mainly estrogen-regulated genes from 10 µg/L. Genes for vitellogenin (*vtg1*) and the aromatase a (*cyp19a1a*) were e.g., downregulated at 48 hpf, and 96 hpf, respectively, whereas the estrogen receptors *esr2a* and *esr2b* were upregulated by fadrozole at 96 hpf. Overall, the initial findings suggest that gene expression is able to provide valuable additional information on endocrine disrupting mechanisms already at an early stage before it manifests in the organism at the apical level.

TH156
Effects of activated carbon in the removal of 17-α-ethinylestradiol (EE2): A reproductive approach using Danio rerio
A. de Oliveira Fermino Arine ; C. Hitomi Watanabe; V. Campos, R. Fracacio, Unesp
Chemicals with the potential to unregulate the endocrine systems of vertebrates and invertebrates have been detected in aquatic environments worldwide, highlighting the need to effective treatment for their removal from environments. Adsorbent materials such as activated carbon, has been shown to be efficient in removing organic and inorganic pollutants. Then, the present study aimed to evaluate the toxicity of the ED (endocrine disruptors) 17 - α - ethinylestradiol (EE2) in the fish *Danio rerio* (Zebrafish), before and after adsorption treatment with carbon activated by pyrolysis. The ability of the activated carbon to remove this water pollutant was investigated by chemical analyzes in gas chromatograph coupled to a mass spectrometer (GC- MS). Then, ecotoxicological tests of biological recovery were performed exposing the organisms to concentrations obtained before and after the adsorption treatment. In fish, the reproductive parameters analyzed were number of eggs per female, eggs hatching rate, and larval viability. The results achieved during the survey are being analyzed and it is expected a EE2 reduction after the proposed treatment. Keywords: endocrine disruptors, 17 - α – ethinylestradiol, zebrafish, carbon activated.

TH157
Malformation of reproductive tracts in male rats given flutamide in utero
Y. Ohta, Tottori University / Agriculture; S. Miyagawa, Okazaki Institute for Integrative Bioscience; T. Iguchi, Natl Institutes of Natural Science
Hypospadias is the most common malformation of external genitalia in males, which may be related to abnormalities of androgen production, timing or receptor function during male sexual differentiation during pregnancy. Fludamide, a potent nonsteroidal androgen receptor antagonist, has been used therapeutically to treat androgen-dependent prostate cancer and as a tool to study male reproductive development. On the other hand, since fludamide is known to induce hypospadias when given pregnant rats, Urogenital organs (reproductive tracts, urethra and external genitalia) were examined in rats given oral administrations of flutamide (45 mg/kg/day) from pregnant days 12 to 22. Urogenital organs dissected out from males (neonate, immature and adult) were fixed in Bouin's solution and cut in paraffin for histological examination. At autopsy, each organ was macroscopically examined. Histological examination in serial sections revealed that a major component of phallus in males given flutamide was similar to that of the control females, although adult males aged 60 days given flutamide *in utero* showed severe hypospadias. Formation of hypospadias was evident after Day 40. Accessory glands, such as seminal vesicles and prostate glands, were differentiated in abnormal position, which were located ventral to the vas deferens coming down along urethra. Vas deferens was connected to vaginal pouch forming between penis and anus, but not to the urethra. The lumen of vas deferens and vaginal pouch was sometimes filled with copious eosinophilic secretions. These abnormal development of urogenital organs was evident in neonates, indicating that flutamide exposure *in utero* induces malformation of androgen-dependent (testosterone or dihydrotestosterone) reproductive organs derived from Wolffian duct and urogenital sinus. This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (24510082) Keywords: flutamide, hypospadias, malformation, rat

TH158
Uncertainties in the assessment of environmental effects of chemicals with endocrine activity – a literature study
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In view of the ongoing discussion on the suitability of current risk assessment procedures for endocrine active substances, the aim of a project funded by the German Federal Environment Agency was to contribute to the evaluation if an ecotoxicological predicted no effect concentration can be derived for chemicals with endocrine modes of action with an acceptable level of uncertainty. Factors that lead to an increased uncertainty in the environmental effects assessment of endocrine active chemicals as compared to baseline toxicants were identified based on a literature search. Data on endocrine effects on fish and aquatic invertebrates were used to support this evaluation. The project focussed on the effects assessment for the aquatic environment according to Appendix 7.8-5 of REACH guidance document R.7b. The following two key factors were identified: (1) The current evaluation of endocrine activity only covers effects on the estrogen / androgen and thyroid axis. For aquatic vertebrates, a tiered testing strategy is available to assess these effects, whereas such a strategy does not exist for other endocrine modes of action. Moreover, effects on invertebrates are insufficiently covered. (2) At present, it is difficult to assess whether the results of tests with few standard test species are protective for all wildlife species. For fish, available effect concentrations in species with similar metabolic capacities are often in the same order of magnitude, while larger differences are observed between species differing in their metabolic capacities. For invertebrates, cross-species extrapolation is far more difficult. This is due to the much higher diversity and heterogeneity of invertebrates and the often fragmentary knowledge on endocrine effects and underlying endocrine processes. The uncertainty of the effects assessment for endocrine active substances is also increased by mixture effects, which include additive effects as well as other interactions. It may be increased if sensitive developmental windows coincide with worst case exposure conditions. The relevance of other factors (e.g. effects on reproductive behaviour, effects with uncertain population relevance and low-dose effects with non-monotonic dose-response relationships) appears to be lower. The specificity of the identified factors for endocrine active substances is evaluated. For some of the identified factors, the feasibility of reducing the resulting uncertainties is addressed.

TH159
Endocrine Disruption: anthropogenic creativity or a hazard of ecological concern?
J. Burke, TSGE LLP / Ecotoxicology
The term "Endocrine Disruption" arose in early 1990's and since that point an extensive compendium of laboratory based research and a significant number of field studies have been conducted to attempt to understand the related mechanisms, modes of action and endpoints that can be affected by chemicals. However, literature searches clearly indicate that substances such as perchlorates were identified to have hormonal effects in fish and amphibians in the early 1970s prior to the anthropogenic creation of the phrase "endocrine disruptor". The effects observed included enlargement and inactivation of the thyroid, arrested sexual development and significant histological changes in the thyroid and pituitary. These effects would now be considered to indicate "endocrine disruption". Since 2012 documents on endocrine disruption have been published by World Health Organisation, European Food Safety Authority and European Commission Joint Research Centre and national authorities. Based on regulations such as (EC) No. 1107/2009 and (EC) No. 528/2012 for plant protection products and biocides, the European Commission was expected to issue regulatory criteria for endocrine disruptors by the end of 2013. However, the commission has delayed action on the issue of criteria, in favour of considering the launch of and impact assessment on the required endocrine criteria and therefore interim criteria as detailed in the REACH regulation (EC) No. 1907/2006 will apply. The amount of documentation that has been published and the change in approach by the commission clearly highlights the scientific and regulatory complexity of this topic. The aim of the presented work is to review public domain data (mammalian, avian and fish) and EFSA conclusion reports for a selection of chemicals suspected of having varied endocrine potency and use this information alongside environmentally realistic field application scenarios detailed within GAPs to (i) assess whether laboratory based studies use excessive concentrations, exaggerate effects and raise unnecessary cause for concern in ecological settings (ii) highlight the importance of ensuring that the term "endocrine disruptor" is used with caution (iii) recommend a default set of endpoints and considerations that should be available and used during the risk assessment of chemicals effecting hormonal systems and (iv) suggest a minor change in the WHO definitions for use in environmental risk assessments.

Sustainability of Swiss Chocolate Production (P)

TH160
Developing inventory data for chocolate: Importance to consider impacts of potential deforestation in a consistent way among ingredients (cocoa, sugar and milk)
S. Humbert, L. Peano, Quantis
Chocolate is definitely a delicious thing! However, some could feel guilty to consume a product that may have contributed to environmental impacts, such as deforestation, in its production. This presentation explores the importance of deforestation impacts in the supply chain or chocolate and the importance to assess this issue in a consistent way among cocoa, sugar and milk. The potential deforestation is based on a share-responsibility approach, meaning on an average deforestation on a country base. To simplify, the composition of chocolate is calculated as 35% cocoa, 35% sugar and 35% milk powder (accounting for 5% waste in the process). Results show that from chocolate perspective, milk powder contributes most to the carbon footprint, followed by cocoa. Sugar is not an important source of carbon footprint. From deforestation perspective, milk powder has anyway a high carbon footprint. Therefore whether the concentrated feed given to the dairy cows to complement their diet based on grass and hay comes from deforestation or not does not change a lot the overall carbon footprint of milk powder. For cocoa and sugar cane, if applying a shared-responsibility approach, contribution from deforestation can double or triple the overall carbon footprint of those products. In summary, for chocolate, if one wants a "sweet", one will have a lower carbon footprint if minimizing dairy fraction and maximizing sugar fraction. There is an increasing demand for LCA applied to food and beverage sector. However, currently a major limitation to do so exist: the lack of consistent, up-to-date, comprehensive and transparent inventory data for food products and processes; there is a need to develop such data to improve the accuracy of LCA in the food sector. In this context, Quantis and Agroscope launched early 2012 the World Food LCA Database (WFLDB) project, in collaboration with, ADEME, Bayer, Swiss Federal Office for the Environment, General Mills, Kraft Foods, Mars, Mondelez International, Monsanto, Nestlé, Syngenta and Yara. The WFLDB will be a comprehensive LCA food database providing detailed, reliable, and transparent high quality life cycle inventory data, including a consistent treatment of deforestation, while being in line with other databases. This presentation shows that, if one wants to make reliable and sustainable decisions in the chocolate sector, it is important to have databases considering, in a consistent way, all types of major environmental impacts such as deforestation.

Applications of innovative passive sampling and dosing (P)

TH193
Bioaccumulation, transformation & magnification possibility of PBDEs, OH- and MeO-BDEs in fresh water
U. Kim, Pusan National University / Department of Civil and Environmental Engineering; H. Jo, G. Joo, Department of Biological Sciences Pusan National University; J. Oh, Pusan National University
We investigated the accumulated concentration and distribution pattern of PBDEs and potential metabolites of PBDEs (i.e. OH- and MeO-BDEs) from seven representative species of fish in Nakdong river, Korea. The homologue/congener distribution pattern and relative composition of parent PBDEs and metabolite BDEs were profiled and tried to figure out the biotransformation or natural occurring possibility with relation to the occurrence of OH- and MeO-BDEs. The potential metabolites concentration of fishes was compared with freely dissolved concentration from SPMD to estimate the bioaccumulation/transformation possibility with considering background PBDEs, OH- and MeO-BDEs existence in the aquatic environment. Relative trophic position was estimated by stable isotope analysis for investigating the possibility of biomagnification and understanding the accumulation characteristics of PBDEs and their metabolites in freshwater food web.

Mercury Biogeochemistry and Policy (P)

TH194
Abiotic Photo-degradation Mechanism of Methylmercury in Seawater
K. Zoh, Seoul National University / Department of Environmental Health School of Public Health; M. Kim, Seoul National University / Dept of Environmental Health; A. Won, Seoul National University
Methylmercury (MeHg) is a highly toxic form of the mercury (Hg) species, and can be accumulated in aquatic food chain. MeHg in natural water is controlled by biotic and abiotic factors. Although numerous studies have demonstrated that MeHg decomposition can be microbially mediated in water and sediment, photo-induced decomposition of MeHg in natural water is known as an another significant pathway to reduce the possibility of MeHg accumulation in aquatic food chain. Dissolved gaseous mercury (DGM, Hg^0) is a major volatile species of Hg in water. DGM production in water is mediated by solar radiation and the reduction of inorganic mercury (Hg^{2+}) in water, and can be affected by other abiotic factors. DGM can be also released by MeHg decomposition, but little research has been conducted on measuring the conversion of MeHg to DGM. Moreover, coastal zone plays an important role in the global Hg cycling not only as a sink for terrestrial derived MeHg, but also as a potential source of MeHg to the ocean. Photo-degradation of Methylmercury (MeHg) is known as the important process in mercury cycling that maintains MeHg at low concentration in freshwater lakes, but less is known about its importance in marine waters. In this study, we investigated the effect of the environmental factors (i.e., light wavelength and intensity, salinity, initial MeHg concentration) and primary water constituents (i.e., dissolved organic matter) on the photochemical degradation of MeHg in seawater. In addition, we assessed the production of dissolved gaseous mercury (DGM) and the photo-degradation kinetics of MeHg in order to understand the mechanism of MeHg photo-degradation in seawater. The results showed photo-degradation of MeHg in seawater under UV irradiation showed the pseudo-first-order reaction kinetics. MeHg photo-degradation rate in seawater was dependent on UV light wavelength and intensity. DGM was produced from photo-degradation of MeHg. MeHg photo-degradation rate and DGM production from photo-degradation decreased with increasing salinity. The photo-degradation of MeHg was also affected by the presence of other water constituents. Our results imply that chloride ion could inhibit MeHg photo-degradation by forming complex with MeHg.

Poster Corner Abstracts

Modelling techniques for future-oriented LCA and forecasting scenarios (PC)

MOPC01
Methodologies accounting for indirect Land Use Change (iLUC) effect: review, assessment and future development
M. De Rosa, Arhus University / Agroecology
The increasing demand of feed, food and renewable products (fibers, alternative fuels) is accelerating the demand of land. Land use triggers numerous effects, such as alterations of the soil and above ground carbon stock, transformation of biodiversity, change in water demand and social effects (hunger, land use rights ecc). Those effects take place both directly on the occupied land and indirectly on land outside the considered system boundaries (indirect Land Use Change effects). The awareness of iLUC effects drove the development of methodologies to measure them and suggest mitigation solutions. At present different LCA approaches and equilibrium models have been proposed. However, due to the complexity of the subject and models uncertainties there is limited agreement on their results and validity. This study evaluates a number of state-of-the-art modeling approaches, assessing their limits and strengths. The main characteristics of the models are grouped in the following categories: methods, impact assessment potential and reproducibility, each divided in subcategories. Those characteristics are then classified according to their completeness, robustness, versatility. The result is a ranking of the models aiming at shining a light on their general validity. In conclusion the study recommends areas where further development is needed to strengthen the reliability of iLUC models.

MOPC02
iLUC scenarios of biofuel consumption in Spain from a consequential approach
D. Garrain, CIEMAT / Energy Energy Systems Analysis Unit; C. De la rua, CIEMAT; Y. Lechon, CIEMAT / Energy Dpt Energy Systems Analysis Unit
The indirect Land Use Change (iLUC) impacts of biofuels relates to the unintended consequence of liberating more carbon emissions due to land-use changes around the world induced by the expansion of croplands for biofuel production in response to the increased global demand. These induced tensions cause changes which affect greenhouse gases emissions, hence climate change impact category. Two different methods are currently available for identifying the effects: i) Economic equilibrium (general or partial) models, or complex approaches that attempt to simulate the mechanisms in numeric terms using macroeconomic and/or biophysical models, and ii) Deterministic models, or very simplified methods that assume that additional biomass production by definition results in additional land use, roughly calculates how much and allocates it to biomass production, where approximate figures or factors are taken instead of the sensitivity of complex models. European Commission has conducted several expert consultations and developed corresponding actions for addressing iLUC component in Life Cycle Assessment (LCA) of biofuels. After the evaluation of approaches by end-2012, a proposal for adapting the European Renewable Energy Directive (RED) 2009/28/EC was presented to consider iLUC, although only with informative purposes. The estimated iLUC values to be added to the life cycle greenhouse gases emissions of biofuels are 12, 13 and 55 g CO_2 eq / MJ in biofuels from cereals (and starch rich crops), sugars and oil crops, respectively. Many comments and amendments to these values have arisen from stakeholders (European governments, researchers, NGOs, etc.), so this issue is currently being debated. This study presents the analysis of several scenarios of iLUC effects due to the marginal extra-consumption of bioethanol (from wheat, maize, barley, sugar cane and sugar beet) and biodiesel (from soybean, sunflower, rapeseed and palm) in Spain from a consequential approach, *i.e.* system expansion was considered to handle co-products. A deterministic model has been applied to actual biofuel consumption scenario in 2011. Results show figures of 20-60 g CO_2 eq / MJ in biodiesel, and 16-60 g CO_2 eq / MJ in bioethanol. The main difference of this large interval is due to the selection of the location of raw materials.

MOPC03
Additional production of ethanol in Brazil in a consequential perspective
R.R. Sallaberry, TU Darmstadt / Material Flow Management and Resource Economy; L. Schebek, Technische Universitaet Darmstadt / Material Flow Management and Resource Economy
Replacing fossil fuels by biofuels is a key mitigation element for reducing human-induced global warming. Not a simple issue, the promotion of biofuels affects diverse economy sectors such as energy supply, transport, agriculture and forestry. One interesting example happens in Brazil, the fifth largest country in the world and in the top 3 of ethanol producers worldwide. An increase in production is expected in order to supply a potential international market as well as the domestic market. A decade ago the introduction of flex fuel vehicles boosted the consumption of ethanol in Brazil. In practice, local consumers have the free choice between gasoline and ethanol, both available in the whole territory. Nevertheless, the increasing demand can be satisfied by a comprehensive efficiency improvement in the ethanol production, even considering the fact that Brazil still has potential for expansion and intensification of agriculture. This poster presents a model of the sugarcane industry for the marginal Brazilian ethanol system, considering the system expansion and the substitution products. The proposition includes the identification of the unit processes that change as consequence of a decision. Furthermore, it should be defined how unit processes are linked, as well as the identification of the scale, trends and changes in the market. The main sources are reports and forecasts from the Brazilian federal government. In order to model the consequences of ethanol use expansion, the chosen methodology is consequential life cycle assessment (CLCA). CLCA represents the convergence of LCA and economic modeling approaches. Besides, it reflects the possible future environmental impact from a change in demand of the product under study. The assumption is an increasing demand of ethanol as a marginal product to satisfy both the domestic market and the international demand until 2020. In order to present the potential improvements, a table will show and classify the bioenergy process chains for Brazilian framework conditions (feedstock - process routes - product combinations). Moreover, the modifications related to the additional production of ethanol and energy will be explored in a consequential perspective. Among others, the modifications are the use of sugarcane straw, ethanol from lignocellulosic biomass, sweet sorghum as feedstock, production of pyrolitic products and new uses for ethanol (ethene, bioplastics, fuel for airplane and motorcycles, mix with diesel).

MOPC04
LCA of future biorefinery systems using the Virtual Sugarcane Biorefinery framework
O. Cavalett, Brazilian Bioethanol Science and Technology Labora; T. Junqueira, M. Ferreira Chagas, E. Morais, A. Bonomi, Brazilian Bioethanol Science and Technology Laboratory CTBE
Considering the low carbon economy context, design of future biorefinery systems (e.g. lignocellulosic ethanol) must account for its potential environmental impacts and proper compre them with fossil based alternatives. Since a lot of uncertanties are associated with in the techical parameters of considered scearios for desing future sugarcane biorefineries, uncertainties on modeling the main LCI parameters are used to provide environmental impacts results in terms of rages using a Monte Carlo simulation. Monte Carlo simulations enable an investigation in to how input uncertainty propagates through the life-cycle emissions model. The focus of this work is to present a set of environmental impacts as ranges and also to raise discussion on the implications of uncertainties in the coparsson current and future sugarcane biorefineries with equivalent fossil systems. Using a streamlined life-cycle assessment for future biorefinery alternative, results showed some probability of greater impact than the distribution for current first generation sugarcane biorefinery but there is always a high probability of future biorefineries present better environmental impacts than fossil fuel based equivalent product. The proposed scenario analysis with environmetal impacts presented as ranges was useful for the decision-context situation where it is necessary to compare between fuel alternatives.

MOPC05
Combining Life Cycle Assessment and economic models for ex-ante evaluation of dairy production systems
T. SALOU, INRA UMR SAS / EA; C. Le Mouel, INRA SMART / SAE; H. van der Werf, INRA UMR SAS / Environnement etAgronomie
Climate change and evolving European agricultural policy will affect the context for milk production in France. Extreme weather events should be more frequent, volatility of prices of agricultural goods could increase. To help producers face these challenges, dairy production systems adapted to this likely future context need to be designed and assessed. They must be efficient regarding both environmental impacts and food production. The aim of this INRA-ADEME PhD project that started in November 2013 is: 1) to identify and characterise promising future milk production systems; 2) to identify political and economic contexts that favour the development of the promising systems identified Seven systems will be studied: three current systems and four innovative systems. First we will compare and characterize the food production potential and the environmental performance of each system using Attributional Life Cycle Assessment (ALCA) identify the systems best suited to the future context. Then, direct and indirect impacts of switching to the more promising systems will be assessed by Consequential LCA (CLCA). The MATSIM-LUCA partial equilibrium economic model currently developed at INRA will help to assess consequences of switching, notably on land-use and land-use change (LULUC). It has been shown that results of CLCA are highly sensitive to the economic model used. To explore this sensitivity, we will compare MATSIM-LUCA to an alternative market and trade model relying on different specifications of both the livestock output supply and the agrifood trade

flows. Finally, we will explore the effects of different economic and political contexts on the performance of the studied systems. Scenarios such involving adverse animal health or climate events, changes in economic policies or environmental regulations will be tested. The poster will present the proposed methodology and the systems that will be compared

MOPC06
Parameterisation of LCI/LCIA models of agricultural systems emissions under future pressures
N. Cosme, Technical University of Denmark DTU / DTUMAN QSA; T.J. Dijkman, Technical University of Denmark / DTU Management Engineering; M. Birkved, Technical University of Denmark
Agricultural production currently faces two important challenges that need to be overcome in the next decades. Firstly, the expected increase of the global human population will put more pressure on productive ecosystems to accommodate the growing need for food. Secondly, climate change as a consequence of anthropogenic emissions is forecasted to increase the pressure on natural and semi-natural systems' productivity through various mechanisms resulting in e.g. an increased frequency of severe weather events or loss of nutrients in soil. An aspect that both the increasing food demand and environmental pressures have in common is the urge to enhance agricultural/food production efficiency, i.e. to produce more (while maintaining an acceptable quality) despite the difficulties raised by climate-driven pressures. Land-based food production is expected to compete with feed/non-food crops, forestry and protected areas for biodiversity, as well as land for bioenergy. Increasing agricultural yield may then be the best option. Accessible ways to rapidly ensure it consists of additional application of fertilisers and pesticides and an increase of their efficiency, while dealing with scarcity of phosphorus. Life Cycle Assessment (LCA) has been dealing with the environmental impacts from emissions and resources consumption from human activities including agriculture. Several approaches for Inventory (LCI) and Impact Assessment (LCIA) modelling of agricultural activities have been published recently. To enhance the agriculture yield by adding nutrients and chemicals, humans will potentially increase the magnitude of the resulting emission flows to the ecosphere. The linearity of the emissions fate and impact modelling suggests the assertion that the more nutrients or chemicals we apply in these systems, the greater the emissions and hence the impacts will be. This consequence will be illustrated by case studies describing how the impacts from fertilizer and pesticide use increase for such agricultural intensification and under future climatic circumstances. Models' sensitivity to the varying parameterisation from e.g. temperature raise or surface runoff (increased rainfall, drought), and the variation range of such inputs will also be addressed. LCA methodologies can provide useful information on the possible and predictable effects and damage to ecosystems and anticipate management and safety practices to minimise ecological, social, and economic impacts.

Delving into the social and monetarised environmental impacts during the evaluation process of the Life Cycle of products in order to be able to take all three pillars of sustainability into account (PC)

MOPC07
Can products be compared in S-LCA as in (environmental) LCA? A pilot of Natura's soap
C.M. Lie Ugaya, Universidade Tecnológica Federal do Paraná / DAMEC; N.T. Haberland, UTFPR; F. Brones,
(Environmental) LCA has been largely used to compare the impacts of products. The aim of the current study is to evaluate if products can be compared in S-LCA using the same technique that is used in (environmental) LCA. To answer this question, Natura, a Brazilian cosmetic company, performed a pilot project in which two soaps were compared: one that includes organic cocoa that is produced by small farmers organized in Cooperatives and the soap without cocoa. In the cradle to gate study, it was applied UNEP and SETAC (2009) for the company's 150 g soap. To establish the product system, all processes that contributed with more than 1% of working hours/mass of the total product was considered. Specific data were collected in five organizations: cocoa production, palm oil production, Natura, that produces the noodle and final pagkaging, the soap producer and the distributor company. Then the Subcategory Assessment Method (SAM) based on Ramirez et al. (2012) was applied. In SAM, organizations are evaluated in four levels: A is given for organizations that upscale the good practice along the value chain, B for organizations that fulfill a Basic Requirement, and C and D depends on the context where the organization is located. At last, an aggregation by working hours along the life cycle stages was performed for each subcategory (Haberland et al., 2013). As the social profile was worse in companies with less organized management, the results of the cocoa soap were worse than the soap without cocoa. Nevertheless, the workers and of cocoa farmers perceived better social conditions, for example, due to the better prices achieved by the organic cocoa than the traditional one, which was not absorbed by the study. In this sense, instead of comparing solely with a product system without the cocoa producers, it was added that the consequences for small farmers in the case they no longer supply to Natura, resulting, for most of the subcategories, that the consumption of cocoa is more beneficial. Haberland, N.T., Ramirez, P.K.S, Petti, L., Brones, F. And Ugaya, C.M.L. (2013) The use of aggregation step in Social Life Cycle Assessment: cocoa's soap case study. CILCA 2013. Ramirez, P.K.S., Petti, L., Brones, F. and Ugaya, C.M.L. (2012). Subcategory Assessment Method for Social LCA: Application for Workers in Natura's Cocoa Soap. UNEP and SETAC (2009) Guidelines for social life cycle assessment of products. Paris.

MOPC08
Stakeholder Analysis and S-LCA: there are elements of overlapping and complementarities?
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In recent years, increasing attention is paid to political actions and public interventions that are established as a result of a share owned by citizens of a nation, by the local population. Social inclusion in decision-making has become a central element also in the preparation of rural development policies of the EU. Similarly, increasing attention is paid to the development of products / services that are produced in compliance with the conditions and social welfare of workers included in the production process. The Stakeholder Analysis (SA) is a method of investigation that, through the systematic collection of qualitative information and their interpretation, allow to understand what are the interests that must be taken into account in the design of a policy, program or any other action, identify the key players and interact with them effectively. The SA allows us to understand the values, interests, aptitudes and aspirations of stakeholders favoring a dialogue between the parties more transparent and coherent. Social-LCA (S-LCA) assesses social and socio-economic impacts found along the life cycle (supply chain, including the use phase and disposal) with generic and site specific data. It differs from other social impacts assessment techniques by its objects: products and services, and its scope: the entire life cycle. Social and socioeconomic aspects assessed in S-LCA are those that may directly affect stakeholders positively or negatively during the life cycle of a product. They may be linked to the behaviors of enterprises, to socio-economic processes, or to impacts on social capital. Depending on the scope of the study, indirect impacts on stakeholders may also be considered. The aim of this study is to identify elements of overlapping and complementarity between these two techniques; explore the possibilities of their use in combination, to improve the robustness of the results during the evaluation process.

MOPC09
Social Life Cycle Assessment applied to housing retrofit: case studies in Brussels-Capital Region
M.I. Touceda, ULB / Polytech service MAT; A. Richard, ULB; M. Degrez, ULB / MAT
Regulatory framework for housing renovation in Brussels-Capital Region addresses two main goals: the regional building code intends to ensure some minimum levels of health, equipment and security. On the other hand, the building energy performance regulation focuses improving the energy efficiency of buildings during their use phase. Subsidies are provided according to these guidelines: to the energy performance level achieved after renovation, as well as to some specific measures such as insulating the building envelope, green roofs or the use of wood for windows frames. Bearing in mind the objectives of sustainable development, some aspects might be missing to be considered within the current subsidies provision system, such as considering the complete Life Cycle of the building. Furthermore, housing retrofit must also address socioeconomic aspects, since the built environment has such an important impact on society. Important advancements have been made for social life cycle assessment: building-specific tools such as LEED or BREEAM are increasingly including social aspects in their methodologies; UNEP-SETAC initiative has developed some Guidelines for products; and the standardisation of methods for analysing the whole building is ongoing by the CEN committee. All these available tools provide us with useful indicators. However, after analysing the specific context of housing renovation in Brussels Capital Region, issues such as affordability of renovation works, fuel poverty, high levels of unemployment and deteriorated working conditions, were identified not to be covered. Some existing indicators have been adapted and some new ones have been developed to propose a specific methodology. The methodological proposal has been tested: case studies are some housing typologies representing the Brussels real state. Some different options representing the most common practices in the local renovation sector have been proposed for each case and their social impacts have been analysed. This comparison allows studying the sensitivity of social performance indicators, in order to check if they are indeed relevant to define policies and subsidies. Results show relevant differences between the social performances of possible options. Therefore, social impacts cannot be disregarded in decision-making processes. Sensitivity assessments provide public bodies with useful information for granting subsidies from a comprehensive point

of view of sustainable development.

MOPC10
Social Life Cycle Assessment: case studies from the textile to the nanotechnology sector
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To include social aspects in LCA is challenging. LCA is a well established method, aimed to quantify use of material and energy of a product from cradle to grave. In social LCA, quantification is not always possible. Social aspects are often controversial, arguable and there are even disagreements in assessment methodologies. However, there is an increased interest from society and pressure on companies to show social performance of the production. Good results in the environmental dimension are not enough, when a product have a “bad reputation” connected to social issues. The Social LCA (S-LCA) is a new method, and as of today, the most accepted one in the LCA community.Some of the few existing examples of S-LCA applications come from existing projects at Østfold Research.The case study of textile production for clothes might imply, besides high use of energy and emissions, social hotspots. These are for instance poor working conditions, child labor, low wages and violation of human rights, during raw material acquisition, fibers and clothing production. A social hotspot database (SHDB) has been developed by New Earth, aiming at making social analysis easier and less time consuming. Ostfold research used this to assess social themes for a specific case study related to three Norwegian clothing design studios. The social topics of “labour rights and decent working conditions” and “health and safety” for the stakeholder category “worker” were accessed. Preliminary results show different social performance in the textile production chain for the countries involved as suppliers and sub-suppliers of material. Another social LCA case study regards the production of bioethanol and biochemicals at a Norwegian biorefinery using lignocellulose from local forests as raw material. In this case, the authors will develop specific indicators for assessing the social sustainability of the production and the creation of value for the society. Further studies concerns the social aspects, including health and safety and ethical issues in the nanocellulose production and use in various applications such as paper and packaging, oil industry and tissue engineering. Social LCA is challenging because of issues like how to measure, evaluate and emphasize the different social topics. At the same time it deals with a variety of important aspects related to value chains ranging from the textile industry to nanotechnology.

MOPC11
S-LCA in agri-food sector: the case study of a sardinian wine
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The aim of this work is twofold: 1) focusing on the importance of the S-LCA for the agri-food sector, 2) present the first results of a case study applied to a wine of great extent integrated operating in Italy, in the north of Sardinia. For the agri-food sector, which is dependent on natural, human and physical resources, responsible innovation is increasingly being viewed by firms as a corporate and strategic necessity to ensure long-term sustainability. Yet the agri-food sector today faces critical challenges: global food demand is due to double in the coming 25 years, requiring an equivalent increase in agricultural production. The growth in demand increases the potential to capture value from agriculture and food production, and could offer large numbers of small-scale producers an opportunity to improve their livelihoods. For this to occur, however, a good share of the value generated by agrifood chains needs to be captured along all the value chain starting from the producer level. So the S-LCA methodology seems to be the best one to address this issues.

MOPC12
Social Life cycle assessment in a managerial perspective: an integrative approach for business strategy
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The attention on social, economic and environmental impacts and on sustainability field by the costumers and the other general stakeholder led businesses to adoption of several tool for sustainable development patterns and, in particular for, social development patterns. Social impacts evaluation is one of sustainability cornerstones of products and services. About that, Social Life Cycle Assessment (SLCA hereafter) focuses on the social impacts of life cycles but, as this is a relatively new analytical approach, no globally shared application tools have been developed yet. The Social Life Cycle Assessment methodology can be described as a tool that shows a strategic and management vision of the social product sustainability. It takes the form of an analysis that lets the company observe the social impact of the product through its sustainability evaluation throughout the life cycle (Benoit C. et al., 2010). The classification of stakeholders is still controversial and not universally harmonized in the various analysis models. There are a common point balance categories: customers, staff, suppliers and the local community (Hinna, 2005; Bowie, 1999; Schwartz, 2006; Sacconi, 2005; T.Donaldson and LE Preston, 1995). The purpose of the study is to analyze the instruments of stakeholder management and Corporate Social Responsibility in order to create a model of integration between the tools of social responsibility, SLCA and Stakeholder Management Approach.

Landscape ecotoxicology and spatially explicit risk assessment of toxicants (PC)

MOPC13
GIS-based Integrated Risk Assessment applied to Taizi river, China
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Natural ecosystems are characterised by a high spatial and temporal variability that influences ecological processes and modifies exposure and effects of chemical pollutants on bological individuals or populations, especially in watersheds. A science based assessment can identify watersheds in poor health and the likely causes of the decline in their ecological condition. In this paper, the GIS (Geographic Information System)-based Integrated Risk Assessment (IRA) methodology developed in the EU MODELKEY project (and implemented in the MODELKEY DSS) is applied to the Taizi river, China, in order to assess the Ecological (ES) and Chemical (CS) Status (according to EU Water Framework Directive) of each sampling site in different habitat typologies . The available dataset is derived by sampling work carried out across the Taizi river catchment for physico-chemistry (i.e. CODMn, TN, TP), chemistry (i.e. Phenols), and biology (i.e. macroinvertebrates, fish, algae), involving 69 river sites sampled in 2009, and 154 tributaries sites sampled in 2010. First results of this on going application are the identification of habitat typologies, reference conditions, LoE-related indicators and thresholds, according to the spatial and temporal evaluation of the dataset required in the MODELKEY DSS. Next application steps will include 1) the ES and CS assessment of each sampling site by integrating the selected LoEs (i.e. Biology, Physico-chemistry, Chemistry and Hydromorphology), and 2) the comparison of obtained results with the water quality assessment conducted in Taizi river catchment in 2012, following a different methodological approach. The obtained results will be reported and discussed in the platform presentation. The comparison between MODELKEY DSS and previous assessment results would make assessor a better understanding on identification of the biological communities that are potentially at risk and the stressors (e.g. toxicants) that are most likely responsible for the observed alterations in Taizi river catchment. Furthermore, comparison will aslo allow a better understanding of strenghts and weaknesses of the two methodologies in addressing specific issues, such as the selection of spatially and temporarily relevant input data, which are crucial for a reliable integrated risk assessment.

MOPC14
Xplicit-IBC-grass - A model for risk characterisation of plant communities
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Non-target terrestrial plants (NTTP), i.e. plants growing near agricultural fields, are potentially impacted by herbicides. Specific Protection Goals (SPGs) defined for NTTP like ‘no decrease in biodiversity’ address community level at the spatial scale of field edges or landscapes. Therefore, model approaches addressing community level endpoints defined in SPGs are gaining greater importance in ecological risk characterization. To analyse community-level risks on landscape scales, we aim to link Xplicit, a mathematical model addressing herbicide exposure and individual-level risk at landscape-scales, and IBC-grass, a spatio-temporal plant community model based on a plant functional type (PFT) approach. This publication focuses on the development of the IBC-grass part of the combined approach.\nBased on published phytosociological data, representative plant communities were modelled in IBC-grass. Public domain databases were used to classify species into PFTs. We selected model parameters potentially affected by herbicide exposure. Thereby we systematically tested a broad range of impact scenarios where either one or all PFTs were negatively affected. Herbicide exposure was assumed to occur during 4 weeks of each simulation year over a period of 10 years and was followed by 15 years without simulated herbicide exposure to analyse the recovery potential. An artificially high level of impact by the hypothetical herbicide was assumed, aiming to produce effects and demonstrate sensitivity of the model.\nDepending on the influenced model parameters, simulated herbicide exposure resulted in various effects on the PFT and the corresponding community, respectively. An increase in resource stress level for all PFTs changed the community significantly with no return to the original community stage within 15 years after final treatment. Similarly, if all selected model parameters were negatively affected by a hypothetical herbicide, there were severe changes both on PFT level and on community level. \nResults of the broad range of tested scenarios clearly demonstrate the potential of IBC-grass to assess herbicidal effects at plant community level. Progress in translation of individual level risks into IBC-grass has the potential to contribute to a more integrated risk assessment approach for NTTP. By integrating spatio-temporally varying exposure at different scales – obtained from Xplicit – into IBC-grass will thus allow to better address future SPG Assessment Endpoints.

MOPC15
Spatially differentiated comparative toxicity potentials of metals in global coastal seawater
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The comparative toxicity potential (CTP) of a chemical, used in Life Cycle Assessment (LCA), represents an estimate of the potential ecotoxic impact caused by a unit emission of the chemical through environmental exposure. The fate of metals in the environment and the exposure to the toxic species of a metal is influenced by water residence time and metal speciation, governed by aquatic environmental conditions, in particular salinity, pH, and DOC concentration. This study developed CTPs for the metals Cadmium, Cobalt, Copper (II), Nickel, Lead and Zinc spatially explicit for 64 Large Marine Ecosystems (LMEs) - independent seas that together comprise the coastal seawater in the world. The waters of the LMEs show a large variation in residence time and temperature, but more modest variations in DOC, POC, SPM concentration, pH and salinity. The CTP is the product of the Fate Factor (FF, representing the residence time of total metal in the seawater compartment), Bioavailability Factor (BF, the fraction of truly dissolved metal within total metal) and Effect Factor (EF, indicating the ecotoxicity of the truly dissolved metal). For each LME, the specific water chemistry was applied to derive two CTPs - one for direct emission to seawater (CTP_{sw-sw}) and one for emission to freshwater followed by transportation to seawater (CTP_{fw-sw}), incorporating metal removal in both freshwater and estuary. The multimedia fate model of USEtox, metal speciation model WHAM 7.0 and Free Ion Activity Model (FIAM) were used in the derivation of FF, BF and EF respectively. Results showed a strong dependence on residence time, but also other coastal water parameters were of importance. Metals thus showed lower CTPs in LMEs with short water residence time and high organic matter concentration. In contrast, the highest CTPs were observed in LMEs with long water residence time and lower organic matter concentration. The metal ecotoxicity ranking given by the CTPs also differed between the LMEs. CTPs ranked Cd the highest in most LMEs. CTPs of each metal varied up to ca. 2-3 orders of magnitude across LMEs, mainly driven by differences in water residence time. The results indicate the relevance of taking emission location into consideration when assessing metal CTP in coastal seawater for LCA.

MOPC16
Seasonal variability in physiological biomarkers in Mytilus galloprovincialis from the Spanish N-NW coast.
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Marine organisms are highly seasonal animals in relation to their physiology which depends, among other factors, on the annual cycle of reproduction. In bivalves, reproductive cycle is regulated by two main environmental factors: temperature and food availability. Bivalve condition, represented as the proportion of tissues related to shell, depends of both: food availability and sexual maturity which are highly interdependent. Food availability and, consequently, reproductive status varies not only with season but also spatially. Integrated monitoring of pollution carried out by the IEO along the N-NW coast of Spain has evidenced that the variability of the environmental conditions produce spatial differences in mussel condition which seems to mask the biomarker responses to pollution. Thus, there is a need to study the natural variability of biological responses used as pollution biomarkers at different seasons and in different habitats in order to establish an adequate link between chemical pollution and biological responses. This study aims to assess the natural variability of physiological responses in the mussel *Mytilus galloprovincialis* in 5 different sites from the Spanish Marine Pollution Monitoring Program which are differentiated in both their natural ecology and their anthropogenic pressure. The potential causes for the seasonal fluctuations in physiological biomarkers are examined in relation to environmental and endogenous factors. Physiological measurements were performed under the same standardized laboratory: clearance rates, absorption efficiency and respiration rates which can be integrated in the –scope for growth- SFG index. Biological characterization of mussels from a biochemical, histological and anatomical point of view was also performed. Physiological biomarkers were clearly influenced by the annual cycle but in a particular way for each site. It seems that biomarker variability inter-sites in each sampling period is higher than temporal biomarker variability in each site.

MOPC17
USE OF SCREENNING BIOTEST TO ASSESS THE TOXICITY OF PERSISTENT POLLUTANTS
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The live organisms integrate the effects of positive and negative effects to the chemical substances as well as the environmental conditions existing during their growth an development. Since they react to the biologically active components contained in the complex chemical contaminations, the biological test provide more direct measurement of toxicity than chemical analyses. When selecting the kind of test and organisms one should consider first of all the character of the environment for which the test supposed to be representative. The selected organisms should also represent the appropriate trophic level and standard methods. The toxicity of soil contamination can be studied with the use of soil microorganism, plants, earthworms, soil arthropods and other different organisms. The current level of knowledge indicates that a lot of contaminants or products of their microbiological processes demonstrates mutagenic characteristics and imposes a potential danger of genetic diseases for future generations, as well as the risk of cancer nowadays. The results of the genotoxicity tests usually are considered as an indicator of mutagenicity and for the prognosis of carcionogenicity. The genotoxic features of chemical substances can be studied with the use of various genetic end points both *in vitro* and *in vivo*. In order to identify the mutagenic and gentotoxic substances a number of test screening procedures have been prepared and assumed.

MOPC18
Evaluation of detoxication ability of humic products on polluted urban soils using TRIAD approach
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The potential of humic products (HP) to remediate polluted soils is known, but the remediating ability of different HPs is not equal and depends on number of factors. The objective of this study was to evaluate the remediating ability of two HP on polluted urban soils using TRIAD approach, and to obtain an integrated index from chemical, biological and toxicological parameters. Soils were sampled from polluted industrial and unpolluted areas of city Kirov. Polluted soil contained heavy metals (Cd, Ni, Pb, and Cr), and unpolluted one was used as a background reference. Two commercial HP were used as remediating agents: Lignohumate (HP-1) and Nanomagnetitehumate (HP-2). Soil samples were mixed with HP in rates 0.0025, 0.01 and 1.00w%, placed in pots and grass mixture was vegetated for 56 days. Sample with no HP was used as a control. At the end of experiment 3 sets of parameters were examined. Ecotoxicological: grass productivity and battery of toxicological tests using *S. quadricauda, D.magna and E.coli*; chemical: heavy metals, CNPK; and ecological: soil respiration and enzymatic activity. The risk indexes obtained from the 3 different Triad disciplines (ChemRI, EtoxRI, and EcoRI) were calculated and combined in order to estimate an environmental risk index EnvRI. Chemical analyses showed that the amount of available species of Pb and Cd significantly decreased in treatments with both HP at high rates, whereas content of Ni and Cr did not change. Certain increase in P and K content was observed for HP-1 and increase of N – for HP-2. For grass productivity different trends were revealed. HP-1 promoted plant growth: biomass exceeded values of unpolluted control. In contrast, under the influence of HP-2 growth inhibition was observed: biomass more than 50% decreased. Ecotoxicological analyses demonstrated positive influence of both HPs for all the test-cultures. Test-functions in polluted soil were 48-70% inhibited. Treatment with HPs completely removed the toxicity at all rates, in some cases achieving the value of unpolluted control. Application of HPs also influenced development of microbial biomass and enzymatic activity. Calculation of EnvRI resulted in values of 0.22-0.30 for HP-1 and 0.25-0.31 for HP-2, being 0.49 for polluted control. The decrease of EnvRI-values allows to conclude, that both HPs give best remediating effect at rates 0.0025-0.01%. Authors thank the Russian Foundation of Basic Research for financial support (grant 12-04-0123a)

Assessing the risk of environmental pollutants on amphibians and reptiles (PC)

MOPC19
Amphibians and agriculture – chemical fragmentation of breeding pond populations
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Decreasing habitat availability as well as habitat fragmentation and environmental pollution by agricultural chemicals are among the major causes for the observed global amphibian decline. It is necessary to understand how anthropogenic land use influences habitat suitability and amphibian migration. This includes migration between different terrestrial and aquatic habitat patches as part of the natural life cycle of amphibian species as well as genetic exchange between populations and colonization of new habitat patches through dispersal. During migrations over arable land exposure to agro chemicals, such as fertilizers and pesticides, is likely. Due to their skin properties, amphibians are highly susceptible for chemical uptake, which can cause lethal or sub lethal effects. We used an expert-based model approach to assess the migration areas of seven amphibian species that considers permeability of the landscape and includes potential pesticide impacts. Additionally, we investigate the migration of adult amphibians from terrestrial hibernation sites to breeding ponds in spring with respect to pesticide applications in vineyards (Rhineland Palatinate) and winter cereals (Brandenburg). We quantified population shares of single amphibian species migrating just before, during and directly after pesticide applications. Our results show a decreasing size of the expected migration areas as well as an increased number of isolated breeding ponds, when potential pesticide impacts were included as migration costs. In our model, the migration areas of *Rana temporaria* decreased by about 35 %, five breeding ponds became completely isolated. We showed that the share of amphibian populations coinciding with pesticide applications can be considerable during spring migration. A maximum was obvious in *Pelobates fuscus where* over 80% of the migrants encountered a single fungicide application in 2008 in winter rape. Recent laboratory studies showed high toxicity of commonly used pesticides to terrestrial amphibians at field application rates. Therefore, field cultivation and in particular pesticide applications may create a sink for populations during terrestrial activity. In combination with reproduction failure and decreased connectivity between habitats as well as populations, pesticides may promote local amphibian extinction.

MOPC20
Testing terrestrial life stages of amphibians – potential test strategies
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Extensive surveys and data comparisons show that the data package generated for pesticides is sufficiently protective to cover aquatic life stages of amphibians. However, in recent years a few studies were published indicating that terrestrial life stages of amphibians may be vulnerable to pesticide applications, which was not necessarily predicted by respective bird and mammal studies. We propose a conceptual approach when and which additional studies may be needed to address the risk of pesticide applications to terrestrial life stages of amphibians, indicate where further research is needed and show options for relevant higher tier testing. The literature data so far indicating a potential hazard are based on studies conducted under 'worst-case' laboratory conditions maximising exposure to sensitive young metamorphs. Such conditions may be used to identify those compounds which are of low risk. However, if effects are seen, this does not translate into a respective impact under realistic conditions. In order to assess the impact to amphibians under more realistic conditions we have investigated technical options for semi-field studies and collaborated in larger field investigations. We propose appropriate methodology and point to potential problems which may arise when running such studies.

MOPC21
Characterisation of the cutaneous bacterial community in the Perez' frog, Pelophylax perezi: diversity and environmental influence
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Synergetic interaction between chemicals and the emergence of opportunistic disease, potentiated by climate change, are of high concern within the amphibian global decline scenario. Amphibians carry a thin and delicate skin, lacking protection, and being highly permeable. Also, as amphibian skin is permanently moist and covered by mucus that is rich in glycoproteins, it constitutes a good substrate for microbial/fungal development and consequently, for disease. However, the evidence of a skin microbial resident community that may confer amphibians a tolerance to environmental stressors has caught a growing attention. Understand the structure and dynamic of this microbiome or how environmental stressors act on this community is needed to elucidate the complex process of interactions to implement better conservation practices. The present study aimed at i) characterising the skin-associated bacterial community of *P. perezi* frog looking at among and within population variation; ii) evaluate the tolerance to chemical contamination of skin isolated bacteria. The outer microbiome of the frogs was characterized by culture independent method (PCR/DGGE) and by assessing the cultivable portion of the bacterial community. Furthermore, to evaluate the effects caused by exposure to chemical contamination in the skin bacterial community, 30 bacterial isolates were exposed to a metal contaminated effluent. Skin swabs were collected from 28 amphibian individuals inhabiting five ponds, one of them a metal-rich contaminated pond and other a brackish water environment. Culture independent method showed a characteristic profile in frogs from contaminated site and that both intra- and inter-population variability exist in skin microbiome. Assessing the cultivable bacteria of the microbiome, microbial concentration per amphibian sample varied within animals from the same pond and between animals from different ponds. Results revealed low bacterial diversity and density (CFU/frog swab sample) on individuals sampled at the metal contaminated site. Isolated bacteria were genetically identified based on 16S rRNA gene sequence. Toxicological assays exposing bacterial isolates to contaminated effluent showed that the percentage of resistant isolates was higher in frogs from the contaminated site. It was also observed that these bacterial isolates are less affected in their growth rate than the others.

MOPC22
Optimizing the Culturing Conditions of the Amphibian Symbiotic Alga Oophila amblystomatis for Toxicity Testing
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The symbiotic alga *Oophila amblystomatis* is known to play an important role in the embryonic development of the Yellow-Spotted Salamander (*Ambystoma maculatum*). The alga is found in the eggs of the salamander, where it is believed to utilize the nitrogenous waste of the embryo while providing additional oxygenation. There is concern that contaminants that are preferentially toxic to algae may impair the symbiotic relationship, and indirectly affect the salamander. To allow for the screening of contaminants, especially herbicides, it is necessary to develop culturing conditions that allow for toxicity testing in a near-standard (e.g. 96-hr tests with standard endpoints like growth rate) and efficient manner. To this end, we sought culturing conditions providing maximum growth rates under asexual conditions as the genus Oophila shows both sexual and asexual reproductive cycles with sexual reproduction involving cell aggregation. After preliminary tests, a modified Bristol's media with ammonium chloride as the nitrogen source was selected. From this selection, nitrogen content, light intensity and temperature were optimized. With this method, we are now able to screen for impacts of contaminants and rank the sensitivity of *O. amblystomatis* relative to other standard test species for the purposes of risk assessment.

MOPC23
How do the uncertainties in the acute aquatic risk assessment differ between fish and amphibians?
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According to the new data requirement (commission regulation (EU) No 283/2013) to the regulation 1107/2009 amphibians need to be considered in the risk assessment even though toxicity tests are not required. The toxicity may be described based on studies from the open literature. The new aquatic guidance document (EFSA 2013) states that a first tier risk assessment for fish achieves the same level of protection for amphibians. This statement is based on a comparison of acute toxicity data for fish with tadpoles. However, the conclusion of a risk assessor for a risk manager should not only be based on a comparison of the toxicity with the exposure under consideration of the protection goal, but also include a characterization of the uncertainties as described in regulation (EC) No 1107/. Part of the uncertainties are covered by the trigger value laid down in the Uniform Principles, which are supposed to cover the variability between species and laboratories. We argue that a comparison of the acute toxicity data does not give the same certainty to the risk manager with regards to the protection of fish then to the protection of amphibians. In the poster we analyze the uncertainties affecting the acute risk assessment of fish and speculate how these may differ from amphibians. By analyzing a large data set used for the authorization of PPP we will draw conclusions about the variability in toxicity for fish (variability between species, formulation, life stages, test set up etc.) and the uncertainties for amphibians.

MOPC24
Identification of endpoints useful to characterize the impact of pesticides on aquatic amphibian stages
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The EU legislation on pesticides establishes that amphibians have to be considered in risk assessment only in terrestrial environments, being aquatic stages supposedly covered by fish-derived data. However, the little information available comparing

fish and amphibian susceptibility does not guarantee such coverage. One limitation is that comparisons are usually restricted to the most common lethal or growth-related responses, while relevant endpoints at the physiological level are often ignored. The aim of this study is to compile information regarding pesticide effects on aquatic amphibians to determine which endpoints are better indicators of pesticide toxicity. Information was retrieved from papers describing effects of currently used pesticides on amphibians. For each record, the exposure concentration was relativized as a function of the active substance toxicity to aquatic vertebrates. The magnitude of the effect was estimated as the proportional change of the response of exposed individuals relative to controls. The measured parameters were classified into major endpoint categories whose relationship with effect and exposure data was tested with a PCA. The analysis of 1274 records showed that genotoxicity was clearly related to amphibian sensitivity to pesticides, while growth, development, immune function and malformations were associated with increased concentration or exposure time. GLMz conducted to explored dose-effect relationships for every endpoint category confirmed genotoxicity as the best indicator (effect vs. concentration: ß=7.380±1.388, χ^2=28.280, P< 0.001; effect vs. time: ß=6.209±1.474, χ^2=17.742, P< 0.001). Moreover, immunotoxic effects were explained by exposure time (ß=9.795±2.942; χ^2=11.081; P=0.001), while oxidative stress biomarkers responded to the exposure concentration (ß=4.695±1.453, χ^2=10.443; P=0.001). Genotoxicity could be a useful indicator of amphibian sensitivity to pesticides but, as it is usually tested only in substances suspected to exert such effect, it requires confirmation in a wider range of pesticides. Functional responses like immunotoxocity or oxidative stress appear as suitable indicators of chronic and acute toxicity, respectively. Further steps shall compare this information based on uncommon endpoints with that from fish species in order to check the appropriateness of surrogates for covering pesticide risks on aquatic amphibians

Advancements in life cycle impact assessment and footprint method development (PC)

TUPC01
Risk and life cycle impact assessment of municipal waste management
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To date, landfilling remains the most common waste management practice in Greece in spite of enforced regulations aimed at increasing recycling, pre-selection of waste, energy and material recovery. In this paper selected alternative scenarios aimed at minimizing the unused material fraction to be disposed of to landfills are analyzed. The aim of is to select an optimum waste management system for the largest Greek cities (Athens and Thessaloniki) by evaluating, alternative waste management systems. The methodological framework of the analysis followed is life cycle assessment, with a special focus on energy and material balance. The environmental impacts assessed are abiotic depletion, global warming, ozone layer depletion, acidification and eutrophication. Public health impacts were also assessed and coupled to the life cycle impacts towards an integrated estimate of the overall pressure exercised by waste management scenarios on both the ecosystem sustainability and population health status. Our analysis points out that landfilling is the worst waste management strategy. At the same time, the investigated options for waste treatment coupled with energy and material recovery would result in very important benefits in terms of greenhouse gas emission reduction. However, not all options are equally benign to the local environment and to the health of the local population, due to non-negligible local emissions. A significant result is the fact that life cycle analysis produces different conclusions than a simple environmental impact assessment based only on estimated or measured emissions. Taking into account the overall life cycle of both the waste streams and of the technological systems and facilities envisaged under the plausible scenarios analyzed herein, alters the relative attractiveness of the solutions considered. Furthermore, waste treatments leading to energy recovery provide an energy output that, in the best case, is able to meet a significant but not high percentage of the urban power demand.

TUPC02
Spatial differentiation for toxic emissions in LCA: the importance of "cumulated equivalent depth" on a 0.5° by 0.5° resolution
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Multimedia and multi-pathways exposure models have been recognized being suited characterization models to generate characterization factors for ecotoxicity and human toxicity impact categories in Life Cycle Assessment (LCA). Given that human and eco-toxicity impacts are highly influenced by the emission location, recent multimedia and multi-pathway model developed spatially differentiated capabilities to reduce model uncertainty and improve accuracy and therefore confidence in LCA results. This work aims at identifying landscape parameters that influence a substance fate and developing an appropriate model to keep a high level of precision while limiting the requirement of a large amount of geographical data. A first evaluation showed that water residence time was a discriminant parameter for persistent substances on a watershed resolution and European scale. This work explores parameters influencing spatial variability on a 0.5° by 0.5° resolution with global coverage in order to capture fine scale and continental specificities. We evaluate the fate of substances with various properties of degradability, evaporation and sedimentation, determine key substance and landscape specific properties influencing their fate, and develop archetypes for freshwater ecotoxicity as a parsimonious surrogate to higher spatial resolution. We observe that the fate of substances with low degradation and important sedimentation and evaporation rate is influenced by the depth of the cell where the substance is emitted and the depth of the following cells. Based on these observations, we introduce a new parameter: the "cumulated equivalent depth" for an emission in a given cell for a given substance. This parameter is substance- and landscape-specific as it depends both on the substances degradation rate and the freshwater residence time to the sea. We developed an archetype model which depends on water residence time and equivalent height of Tinopal. The four archetypes delimitate low freshwater residence time cells (inferior to 1 day), medium freshwater residence time cells (between 1 and 100 days), high freshwater residence time with low equivalent depth cells (below 5 m) and high equivalent depth cells (above 5 m). This preliminary archetype division predicts the fate within 2 orders of magnitude compared to the spatial model. In order to provide scientifically relevant archetype delimitation, an optimized archetype differentiation is planned to be developed in 2014.

TUPC03
Human health impact assessment of indoor pollutants with USEtox in LCA
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Indoor air pollutants are important human health factors, while receiving limited attention in LCA. Depending on parameters like ventilation, room volume and occupation, and other conditions, chemical exposure and health impacts from indoor emission sources are considerably higher than from outdoor emissions. In the context of LCA, available data and methods are limited, reflected by a small number (< 10) of published studies considering indoor exposure, underlining the need for methods that are operational on the level of both the emission inventory and the LCIA. This study aims to bridge practical gaps by providing missing elements (data, assumptions, and recommendations) to operationalise indoor exposure impact assessment in LCA. A major goal was to provide a solution for the usually missing emission data (the "last" main obstacle for application). Applicability was illustrated by a cradle-to-grave LCA case study of a small plus-energy building including furniture, materials, and paints in its interior, comparing human health impacts from indoor exposure during the use phase to "classic" outdoor exposure from all life cycle stages. The functional unit was defined as 2 persons inhabiting the house during 30 years. Determining the quantity of the ~60 volatile organic compounds (VOCs) emitted into the indoor air of the house requires identification of the type and size of the emitting surface of building materials and furniture. The main data source was measured short-term chamber emission data, extrapolated to product life-time emissions by a 2nd-order emission decay model. The life cycle outdoor emissions were modelled using ecoinvent v2.01 and the Ökobau v2 database. The necessary characterisation factors were provided by a beta-version of USEtox implemented with an indoor exposure model expanded by additional fate processes, available via www.usetox.org. This paper shows that indoor exposure impact assessment is operational in LCA and providing important additional insights on human health impacts compared to "classic" LCA, considering only outdoor emissions, typically less significant (by one order of magnitude in this case study) for health impacts. In order to make our indoor emission estimates more transparent and widely accessible, data and models were implemented in a freely available emission inventory tool providing indoor emission estimates for a range of products based on their physical properties and composition, covering about 100 VOCs.

TUPC04
Endpoint method for impact assessment of road traffic noise on human health
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An endpoint method for assessing the impact of road traffic noise on human health is presented. This method is based on previous work done by Franco et al. (2010) (http://link.springer.com/article/10.1007%2Fs11367-010-0213-2). These authors provided a method that relies on publicly available data from strategic noise maps

to calculate the health impact of traffic noise, which is expressed as the number of highly annoyed persons. A fuller development of the above method is proposed here to assess not only highly annoyed persons but also highly sleep disturbed persons, and compute both impacts in DALYs (disability-adjusted life years). The new method is applied here to calculate the noise impact attributable to one additional heavy goods vehicle-kilometre per year on a Spanish motorway. Moreover, the endpoint method ReCiPe is applied to calculate the health impacts due to fuel consumption and air pollutant emissions from the additional vehicle-kilometre. The impacts calculated through the ReCiPe method are also expressed in DALYs, thus allowing the comparison and aggregation of these impacts with the noise impact. Thus, the significance of noise with respect to other traffic pollutants that also cause health damages can be quantified.

TUPC05

Accounting for variation in exposure settings in the life cycle impact assessment of indoor chemical emissions: the case of metal degreasing

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So far, LCIA is primarily focused on the potential impacts of chemicals that are emitted into the ambient environment. However, the life cycle of goods or services also involves indoor exposure in occupational settings or at home. Excluding health impacts from indoor chemical exposure can lead to optimization of products or processes at the expense of the workers' and consumers' health. Hellweg and others (2009) provided a generic framework for integrating indoor exposure to steady state concentrations of air pollutants within LCIA. However, the fraction of the indoor chemical emission that is taken up by the people exposed, i.e. the intake fraction, is determined by a combination of operational conditions (e.g. the volume of the room and the exposure time), and protection measures (e.g. enclosure, local exhaust ventilation or respiratory protective equipment). Therefore, the goal of the present study was to develop an exposure scenario-specific method to estimate the intake fraction of indoor air emissions of chemicals, and to demonstrate the application of the method in a case study on metal degreasing by dichloromethane. We compared the degreasing of 1 m^2 of metal by industrial workers, professional workers, or home consumers. For exposure scenarios with a short duration (1 hour) or protection measures, we found differences up to 1.5 orders of magnitude between our scenario-specific intake fractions and default intake fractions. The intake fraction was particularly influenced by the use of protection measuress. In all exposure scenarios, the life cycle impacts for human toxicity were mainly caused by indoor exposure to dichloromethane (≥70%). Outdoor emissions of dichloromethane contributed up to 22%, while the toxic emissions caused by the production of metal degreaser itself contributed up to 11%. Our findings illustrate that human health impacts from indoor chemical exposure should not be disregarded in LCA case studies. Particularly in the case of protection measures, we recommend the use of our scenario-specific approach.

Effects of Mining on the Local Environment (PC)

TUPC07

Death in the fast lane: Ion imbalance in Daphnia magna exposed in situ in acid mine drainage-impacted waters

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Little attention has been devoted to osmoregulation processes in organisms inhabiting surface waters impacted by Acid Mine Drainage (AMD), where physiological (un)balances of class A metals (e.g. K, Na, Mg, Ca) can be overshadowed by borderline and class B major metal toxicants such as Zn, Fe and Cu , characteristic in AMD. In this study, we evaluated the role and behaviour of these two groups of metals in *Daphnia magna* tissues when exposed to natural waters receiving AMD, using mortality as an endpoint. Daphnids were deployed in environmental chambers at 11 sites along the North Fork of Clear Creek (NFCC) in central Colorado, USA, and whole-body concentrations of Fe, Cu, Zn, Mg, Ca, Na and K were analysed in alive and dead organisms by ICP-OES. Principal Components Analysis identified tissue Ca and in smaller measure Na, Mg and Zn, in this order (associated to the most-downstream stations and the reference station, where survival was close to 100%) and Fe and Cu (associated with the stations directly downstream of the AMD point sources, where highest mortality occurred) as the most influential variables. These associations were corroborated by moderately high positive correlations between mortality and Cu and Fe (0.60 and 0.76, respectively) and moderately high negative correlations between mortality and K and Na (-0.76 and -0.72, respectively). Ca and Mg had low positive correlations with the dependent variable (0.25 and 0.44, respectively), and Zn had an even lower correlation (0.1). Therefore, tissue concentrations of Fe and Cu were good predictors of toxicity to *D. magna* in NFCC, despite Zn having the highest dissolved-metal concentration in the water column. Tissue concentrations of Ca, Mg, Na, and K at high mortality stations were considerably lower in surviving daphnids when compared to the low mortality stations. This is consistent with the general paradigm that exposure of aquatic organisms to metals leads to death by depletion of major ions (Ca, Mg, Na, K). In contrast, tissue concentrations of Ca, Mg, Na, and K in dead organisms at all the stations were similar to water-column concentrations of those ions, therefore suggesting that tissue concentrations of major body ions in dead organisms left in the water column constitute unreliable indicators of mechanism of death.

TUPC08

An investigation of the potential toxicity of dietary Pb to Ceriodaphnia dubia

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There is evidence that dietary metal exposure is readily assimilated by and may cause toxicity to aquatic invertebrates. The effect of dietary Pb to aquatic invertebrates has, however, been rarely investigated. Furthermore, there is no clear understanding of possible interactive effects of dietary and waterborne metal exposure. Here, we studied dietary toxicity of Pb to *Ceriodaphnia dubia* in a chronic reproduction test by comparing waterborne, dietary and combined exposures to a control. For the dietborne exposures the green algae *Pseudokirchneriella subcapitata* was exposed to 6 Pb concentrations (80-340 μg Pb/L) and a control for 64h, after which the algae cells were harvested and fed to *C. dubia* in the diet toxicity test. For the waterborne exposure test media were spiked with 6 different Pb concentrations (80-340 μg/L). The algae used for the dietborne exposure were in equilibrium with up to 52 μg filtered Pb/L and contained up to 3669 μg internal Pb/g dry weight (0.4% Pb on dry weight). We did not observe toxicity within the dietary exposure. The presence of Pb-contaminated diet did also not affect the waterborne toxicity of Pb. These results suggests that dietary toxicity of Pb may be of limited concern in freshwater risk assessment, although comparison with monitored or predicted dietary Pb levels in the field is required to draw a final conclusion.

TUPC09

Metal Distribution and Risk Assessment in Freshwater Sediments under the Influence of Metal Mining.

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Mining industry has adverse effects on surrounding aquatic environments. Because of the accumulation and remobilization of contaminants in sediments, the impacts to water ecosystems can be long-lasting even if concentrations in water are low. In Finland, there are at the moment 52 operating mines and the industry is expected to grow in the near future. Our research is focused on four lakes that are located in the lower reaches of mines in Eastern Finland. The aim of the study is to compare metal concentrations in surficial sediments, pore water and overlying water with toxicity test results and sediment quality guidelines (SQGs) from Sweden and Canada in order to improve risk assessment for mine-contaminated fresh water ecosystems. The sediments were categorized for their metal concentrations based on Swedish sediment quality guidelines. The ongoing study shows moderately high or high concentrations of Cu, Zn, Cr, Ni and As in all of the four sediments and moderately high Cd concentrations in three sediments. Pore water concentrations are low or very low with the exception of one lake, where concentrations of Zn, Ni and As are moderately high. Trace metal concentrations in the overlying water are slightly higher than in pore water, being mostly low or moderate. In one of the lakes, concentrations of Zn and Ni are high. SEM-AVS –ratios were > 1 for two of the lakes. Nevertheless, toxicity tests with *Vibrio fischeri* and growth and reproduction –experiment with *Lumbriculus variegatus* showed toxicity for all of the sediments. In Swedish sediment quality guideline, the risk assessment is based on concentrations and threshold effect -values. Canadian guidelines for sediment are based on sediment concentrations that are derived from studies of field-collected sediments. According to Swedish environmental quality guidelines, all the studied sediments show a growing risk of biological effects. Canadian sediment quality guidelines assess that trace metals in three lakes are frequently associated with adverse biological effects, whereas in one lake, adverse effects are occasional. The lake with highest risks was the same as the one having highest measured concentrations. As a conclusion, all aspects of our studies point to the same direction. The lake sediments are to some extent harmful to environment with two of the lakes posing a higher risk for adverse effects. Nevertheless, based on most of the parameters, the sediments did not fall into the highest risk classes.

TUPC10

Long-term problems in the recovered area affected by the Aznalcóllar's mine spill

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In the Aznalcollar's mine accident, soils were severely polluted by a tailing spill that introduced in depth very high concentrations of heavy metals (Pb, Zn, Cu, Cd) and As. The affected area was rehabilitated during three years by clean-up actions (removal of tailings and upper parts of the soils) and stabilization measurements (amendments and phytostabilization). The original soil use in the area was mainly agricultural and grazing, but due to the persistent residual contamination, the regional government recovered the area into a natural protected area: the Guadiamar Green Corridor. 15 years after the accident, most of the area is showing a good evolution, with generally low concentration of pollutants in very low soluble and available forms. However, serious problems are still detected in the upper part of the affected area (first 10 km downstream from the source of the accident). Nowadays, the lack of remediation measurement, the absence of revegetation and the negative development of the pollution remaining in some sectors, indicate that there is a localized serious risk of contamination. A systematic soil sampling was carried out involving around 100 plots homogeneously distributed along the affected area. These plots are georeferenced and have been monitored over time (1998, 1999, 2001, 2004, 2009 and 2013). The results of the last monitoring show that the pollution concentration and availability indicate levels of concern in many sectors of the upper part of the affected area. These sectors show obvious signs of contamination in field, with the appearance of sulphide oxidation patterns, crust formations and absence of vegetation, occupying areas between 1 and 200 m^2. The analytical data indicate that the mean pH values decrease to 3.50 and the electrical conductivity increases 7-fold in the unrecovered areas in relation to the recovered ones. According to the total concentrations of pollutant, the differences between unrecovered and recovered areas present values of 665.69 vs. 124.91 for Pb, and values of 289.55 vs. 55.02 for As. The study of the total affected surface using aerial images indicate that in some sectors, more than 20% of the area is still unrecovered. Studies regarding potential solubility and availability are carrying out, although these preliminary results evince the need to apply urgent measurements on these sectors to avoid the potential risk of environmental pollution in the area.

TUPC11

Remediation of a metal-arsenic polluted soil using amendments. A case of study in El Arteal mining district (SE Spain).

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In El Arteal mining district (SE Spain), the gradual abandonment of mines and the lack of security measures promoted the increasing of pollution risk spreading associated to trace elements. In order to fight this problem, a metal-arsenic polluted soil from sulphide-mine waste was collected and treated, in all possible combinations, with two different amounts of marble sludge (MS), compost (CM), and byferrox (BF). These amendements were mainly composed by calcium carbonate, compost and iron oxide respectively, and they are widely used in soil remediation. The effectiveness of amendments in the trace-elements immobilization was evaluated with bioassays using different development stages (germination, emergence and establishment) in lettuce (Lactuca sativa L.). After 14 weeks, established lettuces were carefully removed from the pots, measuring the porportional dry weight respect to the control and the foliar trace elements concentration. Soils were analysed using a sequential extraction procedure. The effectiveness of the amendments in reducing the toxicity of the contaminated soil was different depending on the bioassay used. All seeds germinated in pore water from amended soil, and root elongation index (REI) showed significant differences between treatments. Toxicity in pore water was mainly decreasing by marble sludge application. However, only lettuces sowed in soil amended with higher proportion of compost (6%) were established, with significant differences for plant length and weight. Taking into account the different stages of plant development, a comparison of different bioassays is needed before evaluating the effectiveness of amendments to reduce soil toxicity. Regarding foliar cocentrations, lettuces cultivated in amended soil showed higher values than control lettuces cultivated in a natural no contaminated soil. No treatment was able to decrease these levels below concentrations found in bibliography for healthy lettuces. Foliar concentrations were inversely related with hydroxilamine and hydrogen peroxidase extracted fractions, and directly related with acetic acid extracted fraction. The combination of three amendments at higher application rates was the most effective to reduce trace elements bioavailability. Although this treatment was able to reduce negative impact of pollutants in soil, it was not enough to restore the functions of this highly polluted soil.

Fracking, seismics and spills: Environmental risk assessment of oil and gas exploration and production (PC)

TUPC13

Scenarios for shale gas development in the Baltic Basin, Northern Poland

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Shale gas is already being widely used as an alternative energy source to conventional natural gas in the US. Currently, exploratory drilling for shale gas is being undertaken in the EU, but commercial scale exploitation remains a topic of much debate. We developed a methodology to assess the suitability of land for shale gas extraction in Europe, based not only on the resource availability and geology, but also on an array of other influencing parameters, such as the availability of water, accessibility of the site to roads and pipelines, and distance from residential and from protected areas. In doing so, we make a first attempt at assessing possible conflicts with existing and projected land and water requirements. We also carried out a screening level risk assessment of the chemicals used in fracking water. Taking the Baltic Basin in Northern Poland as our study site, we test two technological and two legislative shale gas development scenarios. These scenarios were based on an extensive literature review of the potential physical and legislative parameters which would have to be considered in order to further develop shale gas resources. The scenarios were run in 5-year steps for the period 2013 to 2028. The resulting maps of shale gas well pad allocation were used to assess the impacts on land and water use. Our analysis shows that the impact in terms of land and water consumption varies substantially depending on the rate of development of the resources and the legislative restrictions which may be applied. However, at a local scale there may be non-trivial competition with existing sectorial land and water demands. For example, the land required for shale gas extraction as a percentage of total land converted to industrial purposes within our study area ranges between 19 and 38%. The screening-level evaluation highlighted that several chemicals may pose ecosystem and human health risks. However, impacts may vary significantly according to spatial and temporal aspects, and the evaluation should be carried out on a site-specific basis. Keywords: Land use modelling, shale gas, environmental impact

TUPC14

Bioanalytical and Chemical Assessment of Coal Seam Gas Associated Water

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Coal seam gas (CSG) mining has recently developed rapidly in Australia and the associated water is a by-product that is pumped out of coal seams in order to release CSG. Coal seam gas associated water (CSGW) is generally considered water of poor quality and thus the management and potential further usage of CSGW has been subject of concern. CSGW has a number of potential uses such as irrigation, coal washing for coal mining, cooling in power stations etc. Treated water has been discharged into local rivers or re-injected into underground aquifers. However, only limited studies focus on comprehensive hazard assessment of the quality and quantity of organic micropollutants in CSGW. Conventional chemical monitoring programs have been criticised as these do not account for the combined effects of mixtures of chemicals. In the case of CSGW, this is a particular problem because the identity of organic micropollutants is often unknown, in addition the suite of inorganic pollutants and heavy metals that may also be present. Bioanalytical tools have been used in a wide range of water sources from sewage to recycled water to complement chemical analysis for cost-efficient risk assessment. Here, we are reporting for the first time the application of bioanalytical assessment of three CSGW samples taken from private wells tapping into the same coal measures from which CSG is being extracted in nearby CSG wells. Sampling was conducted in March 2012 and a re-sampling campaign in November 2013 to study the temporal trend. Baseline toxicity levels were comparable that found in secondary treated effluent and clearly higher than conventional surface waters. Induction of arylhydrocarbon receptor was observed in one sample where polycyclic aromatic hydrocarbons were also detected by chemical analysis. Slight estrogenicity was detected but the level was similar to what was found in other groundwater samples, and other endocrine endpoints were below the detection limits. No genotoxicity was detected and the oxidative stress response was very low. Further experiments are ongoing. The outcome of the project will improve the understanding of CSGW toxicity in order to enhance risk assessment and better inform water management decisions.

TUPC15

Total Dissolved Solids Reflective of Natural Resource Extraction Activities Stimulate Growth and Toxicity of the Invasive Harmful Alga Prymnesium

parvum
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Prymnesium parvum harmful algal blooms (HABs) have become an emerging threat to inland freshwater systems due to salinization of surface waters resulting from anthropogenic influences such as natural resource extraction activities (NRE) including hydraulic fracturing (HF; Brooks et al 2011). Previous research from our team has identified salinity as being a critical parameter influencing *P. parvum* HAB dynamics in inland waters (Baker et al 2007, 2009); however, the effect of site-specific ionic constituents is not well understood. Elevated site-specific total dissolved solid (TDS) concentrations of ionic constituents associated with produced waters from these practices may increase the threat of *P. parvum* to inland waters by affecting HAB distribution, formation, and toxicity. In this study, we assessed the effects of TDS concentrations reflective of HF practices on *P. parvum* HAB dynamics. Treatment levels of environmentally relevant HF constituents and stoichiometries in produced waters from Marcellus Shale were selected at varying TDS concentrations. This experiment was designed to assess the effect of HF contaminants at varying TDS concentrations (130, 1000, 4000, 8000 mg/L) and various levels of nutrient limitation (F/2, F/4, F/8) on *P. parvum* growth and toxicity to *Pimephales promelas*. Artificial sea water at 2400 mg/L served as a control (Valenti et al 2011). *Prymnesium parvum* cell densities were monitored throughout each study and exponential growth rates calculated. Results (Figure 1) showed that increased ionic constituents associated with HF NRE stimulated *P. parvum* growth in a dose dependent manner with the maximum densities occurring at the 8000 mg/L TDS treatment level. Conversely, ionic constituents associated with HF NRE did not stimulate *P. parvum* growth at the 130 mg/L treatment levels. Future studies are necessary to develop predictive models of environmental conditions leading to harmful *P. parvum* HABs in regions experiencing intensive NRE.

TUPC16
Modelling effects of oil constituents on survival and reproduction of aquatic species
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Quantitative information on the effects of oil on ecological relevant endpoints, such as survival and reproduction of organisms, is important for risk assessment. Few experimental studies have assessed these effects and, until now, a model quantifying accumulation and effects of oil constituents is lacking. The main goal of the current study was therefore to develop a generic model to estimate the accumulation of oil constituents in aquatic organisms and to estimate the corresponding effects on their survival and reproduction. Using the OMEGA bioaccumulation model, we estimated the body burden (BB) of oil constituents in aquatic organisms. Next, we used the estimated BB and experimental single-species toxicity data for oil and narcotic chemicals from the literature (concentration-response curves, lethal-sublethal ratios) to estimate effects on survival and reproduction. In addition, the majority of oil constituents are expected to exhibit a narcotic toxic mode of action (TMoA), but several oil constituents can exhibit a more specific working mechanism, like activation of the aryl hydrocarbon receptor (AhR) and phototoxicity. Therefore, our second goal was to evaluate the applicability of standard narcotic toxicity values in the effect estimations of oil constituents. We made a distinction in single-species toxicity data between narcotics and oil constituents with a non-specific and an expected specific TMoA. Overall, little differences were observed between the generic parameter values for narcotics and the calculated averages for the single-species toxicity data of oil. The average lethal body burden for oil constituents with an AhR-toxicity and phototoxicity were similar to the average LBBs for oil constituents with a non-specific TMoA. We therefore used the average parameter values for oil to estimate the effects of oil constituents on survival and reproduction and compared our predictions with available literature data. As an example, we present the survival of the amphipod *Hyalella azteca* exposed to fluoranthene. Mortality due to fluoranthene was accurately predicted for the highest concentration (250 μg/l) but was underpredicted for the 62.5 μg/L and 125 μg/L treatments. However, considering that the OMEGA bioaccumulation model requires limited data input and that general oil toxicity values were used to predict the effects of pyrene, it can be concluded that this approach shows potential for the data sparse risk assessment of oil.

TUPC17
Factors Affecting Ecosystem Resilience and Recovery Associated with Oil Spills - Considering Acute and Chronic Effects
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Resilience is the measure of the persistence of systems and of their ability to absorb change and disturbance and still maintain the same relationships between populations or state variables. Ecosystem recovery is the measure of the ability of an ecosystem to recover back to its original condition or steady state. When a species or ecosystem returns to its baseline state, it is termed to be resilient. However, if the severity of a release induces a shift in the population or community structure and it does not return to its baseline condition, or takes a long time to return to its baseline condition, it may not be considered to be resilient. Projecting species resilience and recovery can play an important role in oil spill response decisions. Resilience and recovery can be informed by the development of "risk recovery curves" based upon the acute risk curve, potential chronic exposures, projected recovery trajectories and baseline or projected no-action condition. These curves can provide insight as to the resilience of a particular ecosystem or species and include uncertainty. The incorporation of methods to quantify ecological services into the development of these curves will be discussed. There are many factors that can influence the shapes of these curves for a given event such as the type of oil released, biodegradation rates in the area of the release, presence of recolonisation sources, the breeding characteristics of affected species, the age distribution of the species, the area impacted, the severity (amount) and duration of the release, response options implemented, plus others. This paper will discuss the factors affecting the shapes of these curves and associated uncertainty. We consider curve development for the acute and chronic toxicity aspects of spilled oil for the purposes of supporting decision-making related to oil spill response options during spill events and their potential effects on ecological services.

Ecological Consequences of Exposure to Pharmaceuticals: From the Laboratory to the Field (PC)

TUPC19
Consumption based modelling of pharmaceuticals to predict environmental loads
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Numerous studies describe the presence of pharmaceuticals in the water cycle. However, only few studies address transformation products few studies relate concentrations to pharmaceutical consumption. In the current study 45 pharmaceuticals and 18 transformation products were studied in the river Meuse at the Belgian-Dutch border and four regional streams that contribute to the Meuse in the southern part of the Netherlands. The streams originated from Belgian, Dutch and mixed Dutch and Belgian basins. In total, 24 pharmaceuticals and 13 transformation products were observed in the samples. Observed summed concentrations of pharmaceuticals and transformation products in water ranged from 3.5 to 37.8 μg/L in surface waters. Metformin and its transformation product guanylurea contributed for 53 to 80 % to this concentration, illustrating its importance on a mass basis. Data on the discharge of different streams and demographics of the basins enabled to calculate daily *per capita* loads of pharmaceuticals and transformation products. These loads were linked to consumption of pharmaceuticals in the basin. Simple mass balance modelling accounting for human excretion and removal by waste water treatment plants revealed that consumption could predict actual loads within a factor 3 for most pharmaceuticals. Streams that originated from Belgium and mixed Dutch and Belgian basins revealed significantly higher per capita loads of pharmaceuticals (16.0 ±2.3 and 15.7 ±2.1 mg/inhabitant/day, respectively) than the Dutch stream (8.7 ±1.8 mg/inhabitant/day). Furthermore, guanylurea /metformin ratio was significantly lower in waters originating from Belgium than from the Netherlands, illustrating that wastewater treatment in Belgium is less efficient in transforming metformin into guanylurea. In summary, the current study illustrates that consumption based modelling is suitable to predict environmental loads (and concentrations). Furthermore, different consumption patterns and treatment efficiency are clearly reflected in the occurrence and loads of pharmaceuticals in regional streams.

TUPC20
Environmental Risk Assessment of Metformin
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Metformin (CAS 1115–70–4, Metformin hydrochloride), a legacy antidiabetic drug, has high usage in both North America and Europe and has therefore become the subject of regulatory interest. A pharmaceutical industry working group formed to investigate environmental risks of metformin. Environmental fate and chronic effects data were collated across the industry for the present environmental risk assessment. Predicted environmental concentrations (PECs) for metformin were modeled based on documented usage for the USA with the PhATE model and for the European Union with the GREAT-ER model. These PECs were compared with measured environmental concentrations (MECs) for both the USA and EU. Predicted no effect concentrations (PNECs) for metformin were derived by deterministic procedures, applying an assessment factor of 10 to the lowest no observed effect concentration (NOEC) from chronic studies with algae, daphnids and fish. Both the PEC/PNEC and MEC/PNEC risk characterization ratios showed no significant risk for metformin.

TUPC21
Fish toxicity of diclofenac: ocular lesions and quantitatively evaluated histopathological organ changes
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The non-steroidal anti-inflammatory drug diclofenac is a pharmaceutical agent often detected in surface waters. It is mainly discharged into the aquatic environment via municipal sewage treatment plants. Diclofenac is listed in the watch list of the Directive 2013/99/EG. Currently it is controversially discussed in the literature, if environmentally relevant concentrations of diclofenac can lead to alterations in fish. The present study was designed to enable a realistic risk assessment of this agent based on objective quantitative effect data. In an exposure experiment a total of 120 subadult rainbow trout *(Oncorhynchus mykiss)* were exposed over 28 days (based on OECD Guideline 204) under flow-through conditions to different concentrations of diclofenac sodium (CAS 15307-79-6). Additional 20 rainbow trout were not exposed to diclofenac sodium and served as controls. Diclofenac sodium test concentrations were 0.1, 0.5, 1, 5, 25 and 100 μg/l. A solubilizing agent was not used in this study. Following the exposure, the fish were examined by pathological, hematological and clinical-chemical methods. In contrast to other studies in which histopathological alterations caused by Diclofenac were assessed qualitatively and semi-quantitatively, in the present study morphometric/stereological methods were applied for the first time to quantify compound- related histopathological changes in fish. Pathological alterations occurred primarily in gills, trunk kidney and eyes. Significantly decreased prostaglandin concentrations in the plasma were found already at a test concentration of 0.5 μg/l. Organ alterations were detected in all test groups with exception of the 0.1 μg/l group. In the present study the no observed effect concentration (NOEC) of diclofenac was 0.1 μg/l. Effects of diclofenac exposure on test fish were already observed at concentrations which must be considered as environmentally relevant with regard to published analytical data indicating diclofenac concentrations in surface waters up to 1.2 μg/l (Ternes, 1998). A major advance compared to previous studies is the integration of morphometric/stereological methods to quantify histopathological changes. These methods allow an objective evaluation of histopathological alterations.

TUPC22
Behaviour of diclofenac and structural related non-steroidal anti-inflammatory drugs (NSAIDs) in nitrifying lab scale batch reactors
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Non-steroidal anti-inflammatory drugs (NSAIDs) are a chemically heterogeneous large group of drugs used primarily to treat inflammation, mild to moderate pain, and fever. After their use in human medicine, 30-90% passes through humans completely unchanged. They then reach the surface waters via hospitals and municipal sewage. Compared to the amount of data dealing with the distribution of PhACs residues in the environment, very little evidence has been published as regards metabolic pathways in complex microbial communities like those encountered in the aeration tank of the activated sludge treatment. In a previous work nitroso (TP324) and nitro (TP340) derivatives of diclofenac (DCF) were tentatively identified in the same set-up related in this work. The present study aimed at investigating still uncovered aspects in the environmental fate in WWTP of the NSAIDs such as diclofenac, meclofenamic acid, flufenamic acid, tolfenamic acid, and lumiracoxib. In order to evaluate the biodegradability of the target compounds, the no chlorinated structurally related compound 2-anylinophenylacetic acid (APAA) was also included on the list. Biodegradation experiments in batch-reactors loaded with mixed liquor demonstrated that similar degradation profiles were observed for meclofenamic acid, flufenamic acid, tolfenamic acid and fenamic acid, while APAA degraded faster than DCF. After one day, 50% of APAA (228 Da) was degraded and the formation of one transformation product was confirmed. Structure elucidation by means of ultra performance liquid chromatography-electrospray ionization-hybrid Q ecxactive-MS tentatively identified it as corresponding to nitrosation of the hydroxyl group in the carboxylic acid moiety (TP256). Although the contribution of nitrifying bacteria to the biomass in the mixed microbial community of the activated sludge tank in WWTPs is less than 5 %, the operational conditions of the lab-scale reactors were favorable for the growth of nitrifiers in terms of oxygen supply, and temperature and pH of the mixed liquor. The present study aimed at investigating still uncovered aspects of the fate of the NSAIDs in WWTP

TUPC23
Evaluation of biological endpoints in crop plants after exposures to non-steroidal anti-inflammatory drugs
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The continually expanding global population and the aging demographic of many nations has driven increases in pharmaceutical demand over the last decades, and this rising demand is set to continue. Consequently the presence of pharmaceuticals in the environment is now ubiquitous. This is of concern as these compounds can have deleterious impacts upon organisms, and there is evidence that they may enter the human food chain via drinking water, or crop plants, that have been exposed to biosolids amended soils or contaminated irrigation water. The recent inclusion of pharmaceuticals on a "watch list" (Water Framework Directive; 2013/39/EU) reflects these growing concerns. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is one of the substances included on this watch list. As a class of drug, NSAIDs are one of the most importantly and heavily used medications worldwide. Two exploratory studies aimed to investigate the effects of different, but structurally related NSAIDs on two higher plants, radish (*Raphanus sativus*) and lettuce (*Lactuca sativa*). Plants were exposed to NSAIDs from the fenamic acid (meclofenamic acid, mefenamic acid and tolfenamic acid), acetic acid (diclofenac) and propanoic acid (naproxen, ibuprofen) groups. A number of biological endpoints, alongside impacts upon plant photosynthetic efficiency, and daily developmental progress were evaluated. Initial results show significant differences between a range of treatments and controls. Providing an insight into the potential impacts of pharmaceuticals upon plant growth is particularly important when considered in light of the need to feed an ever expanding population, and dispose of their waste, while maintaining adequate food security.

TUPC24
Genotoxicity of two pharmaceuticals in zebra mussels hemocytes and sperm cells, after ex vivo and in vivo exposure
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The growing global consumption of drugs by humans in association with a deficiency of water treatment plants to totally eliminate them resulted in the contamination of aquatic environment by pharmaceutical residues. Today, our knowledge on ecotoxicity and biological effects of these organic pollutants on aquatic biota is still scarce. Our survey focused on the evaluation of the genotoxicity of two pharmaceutical compounds: diclofenac and carbamazepine, a non-steroidal anti-inflammatory and an anti-epileptic agent respectively, which are frequently measured in surface and ground-waters at concentrations below 10 μg/L. The measure of the DNA damage by the comet assay (or SCGE assay) was studied in the zebra mussel (*Dreissena polymorpha*) as biomarker of genotoxicity. This assay was applied on two cell types: hemocytes and sperm cells after short term *ex vivo* exposures (1-6h) in laboratory experiments to the two tested contaminants and after a long-term *in situ* exposure (2 and 5 months) of mussels in mesocosms contaminated with diclofenac. Concentrations tested for both experiments (laboratory/mesocosms) were environmentally relevant (from 0.1 to 10 μg/L). Results showed a significant increase of DNA damage in hemocytes (after 6h) and sperm cells (from 1h to 6h) at all concentrations tested after *ex vivo* exposure to carbamazepine. No genotoxic effect of diclofenac was observed on hemocytes but a low genotoxicity in sperm cells after 1h and 3h exposure, with a return to a baseline level of DNA damage after 6h (no cytotoxic effect was observed in parallel). However, long-term exposure of mussels in mesocosms revealed the genotoxicity of diclofenac for both hemocytes and sperm cells, with a positive relationship in the degree of DNA damage for the two cell types. Our study is the first to reveal in the zebra mussel the interest to evaluate DNA integrity in sperm cells. Sperm cells appeared as a sensitive cell type with a faster response to a genotoxic stress than

hemocytes. A possible repair of DNA damage by sperm cells was observed, but seems rapidly limited with the intensity of the genotoxic stress. Our results will be discussed according to the litterature data as well as mechanisms underlying genotoxicity of these pharmaceuticals, which still need to be elucidated.

Policy assessment in an integrated systems perspective: indicators and targets to ensure operating within safe planetary boundaries (PC)

WEPC01
Taking a life cycle approach for sustainability assessment of energy policies. A case study of Ecuadorian electricity generation system
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Life cycle thinking has been considered by Ecuadorian government to play a principal role in the definition of sustainable policies on energy efficiency. Appropriate and representative Life-Cycle Inventories are essential for Life-Cycle Assessments quality. It is remarkable that due to the prevalence of hydropower on Ecuadorian electricity mix, the frequently used LCIs are not representative of the Ecuadorian conditions. In a previous work, 14 Ecuadorian power plants (responsible for producing 70% of Ecuador's electricity consumption) were analysed to estimate LCI and environmental burdens associated to 1kWh of electricity generation. The aim of this work is to provide a good understanding of the environmental consecuences of electricity mix expansion plan, analysing the future scenario. The main goal of the present work was the development of a LCI that is representative of hydroelectricity generated in Ecuador, to be used as a basis to estimate environmental burdens of the future electricity grid. A comprehensive LCI was developed considering power plants under construction. The preliminary Work Item on "Framework for the sustainability assessment of civil engineering works" targeted to cover the specific principles and requirements for the sustainability assessment of civil engineering works was taking into account as a methodological reference. The impact assessment was conducted using the CML 2000 methodology. The results obtained show magnitude adequacy compared with previous studies, with a high contribution from the huge civil works associated to the dam construction. The environmental profile reveals a better performance of big hydropower compared to small plants. The sensitivity analysis shows the major importance of the time horizon definition. The most important life-cycle hotspots are associated to the cement and steel life-cycle as well as the operation of civil construction machines. The contribution of reservoir filling is also significant. LCA approach has proved to be a valuable tool to analyse main environmental burdens of hydropower, but important sustainability aspects -such as population displacement- cannot be accounted. The need to provide a general picture for decision makers leads us to establish a collaboration with ecological economists and intangible patrimony experts. A multicriteria analysis is now under development including economic and social aspects.

WEPC02
Multi-criteria Decision-making for Life Cycle Sustainability Assessment: A Case Study of Bioethanol
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Life cycle sustainability assessment (LCSA), as an emerging tool for stimulating sustainable development and progress in sustainability, faces more and more challenges. The most severe problems are: (i) how to make correct decision on selecting the most sustainable technology, pathway or policy among various alternative scenarios due to the concerns of multiple criteria and qualitative criteria for sustainability assessment; (ii) how to address and implement social life cycle assessment that concerning qualitative criteria. The objectives of this study consists two parts: (i) propose a framework for life cycle sustainability assessment by integrating life cycle assessment (LCA), life cycle costing (LCC), social life cycle assessment (SLCA); (ii) develop a multi-criteria decision-making methodology for determining the most sustainable scenario for the decision-makers/stakeholders. LCA, LCC and SLCA are employed to correct the data of the criteria in environmental, economic and social aspects, respectively. Meanwhile, a novel SLCA method for quantifying the criteria in social aspect is developed, and the decision-makers/stakeholders are allowed to use linguistic terms to assess these criteria, fuzzy theory has been used to transform the linguistic variables into real numbers. After the determination of the criteria of the three main pillars of sustainability, Analytic Hierarchy Process (AHP) and VIKOR method are combined to rank to sustainable sequence, and AHP is used to determine the weights of the criteria that are needed in VIKOR, then VIKOR is employed to determine the sustainable sequence of the scenarios. An illustrative case about three alternative scenarios including wheat-based, corn-based and cassava-based for bioethanol production has been studied by the proposed method, and the prior sequence based on sustainability in descending order is cassava-based, corn-based and wheat-based.

WEPC03
The role of the three cultural perspectives in LCA for policy making – human health impacts of future electricity production
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Life Cycle Assessment (LCA) can provide valuable inputs to decision makers for getting an insight to environmental impacts of products or services. However, communication of the results of an LCA can be a challenge, given the broad range of impacts included and value choices made. Despite of these difficulties, LCA practitioners should not refrain from informing the decision makers on the assumptions behind the results. This case study aims to give an example of how to present LCA results possibly used in the planning of future energy policies. It quantifies human health impacts (HHI) of *base-load* electricity production in 2030 in Europe with the LCIA method "ReCiPe" and compares the results of calculations with the three available cultural perspectives "Hierarchist" (H), "Individualist" (I) and "Egalitarian" (E). It is shown by all perspectives that electricity from nuclear and renewable power induces lower HHI than electricity from hard coal and lignite. However, the individual ranking of the technologies may change between the perspectives. With (E), natural gas power is in the range of N&R, whereas it is comparable with the HHI of hard coal or lignite plants with Carbon Capture and Storage (CCS) installed in the (H) and (I) perspective. The benefit of CCS itself is differently judged by the three perspectives, with the (E) perspective showing that total HHI can even increase in plants with CCS compared to plants without CCS. Further, the egalitarian perspective results in general in much higher total HHI – up to a factor 160 between the (I) and (E) perspective. It is also shown that the (E) perspective emphasizes HHI of electricity production due to human toxicity effects, whereas climate change and particulate matter effects dominate the (H) and (I) perspective. Decision makers should be given the opportunity to see such results and understand the foci of the different perspectives. This way, the potentials of LCA are better exploited, which gives them the opportunity to decide actively on trade-offs and to integrate the LCA results in a broader picture towards sustainability.

WEPC04
Agent-based modelling for LCA of electric vehicles deployment policies: what are the key drivers?
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Battery-powered electric vehicles (BEVs) are often presented as a way toward cleaner transportation. As a consequence, European policies aiming at deploying BEVs are flourishing: Luxembourg set an ambitious objective of 40,000 BEVs by the year 2020. To achieve this target, three policy measures were decided: i) 5,000€ incentive for buying a BEV and subscribing to a renewable energy contract; ii) large scale deployment of charging infrastructure; iii) public awareness increasing. Nonetheless, it is unknown how far these measures will help in achieving the objectives and their environmental consequences have yet to be assessed. Moreover, when the Life Cycle Assessment (LCA) practitioner tries to answer these issues, only attributional LCA with restrained spatial and temporal scope and hypotheses are found in literature. In order to tackle these issues, we propose to couple an Agent-Based Model (ABM) to LCA approach to assess the impact of Luxembourgish policies. Because of the high number of cross-borer commuters in the country, the French neighbouring region, Lorraine, was included as well. The ABM developed uses a synthetic population of drivers who calculate every day their own daily agendas and moves their car accordingly. Every month, the technical and economic environment is updated and the agents can sell their cars and buy new ones. Thus, the ABM allows assessing the evolution of the fleet for a given time period and the travels of agents. Finally, the ABM is coupled to an LCA model, which calculates the impacts of new cars production, use and end of life. Results show that the main factors fostering the development of BEVs are infrastructure deployment, car lifetime and BEV attractiveness. They also show that those factors do not have the same influence considering Luxembourgish or Lorraine drivers. Moreover, preliminary LCA results show that the environmental consequences of these policies are complex and could not have been found using an attributional LCA approach. For instance, the deployment of charging infrastructure leads to a higher number of BEVs in circulation but also to electricity consumption at work, which does not necessarily come from renewable sources. The combination of ABM and LCA was proven to be effective for policy support and assessment by accounting for complex interactions between agents and multiple external variables and by assessing complex environmnetal consequences of medium to large scale policies.

WEPC05
Hybrid eMergy-LCI and network's graphs analysis to inform on the sustainability of life cycle activities
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One of the most limiting factors to assess environmental sustainability in large supply-chain networks is the tendency to oversimplify the system's modelling, leading to the loss of relevant information. Essential but challenging is also identifying appropriate techniques to characterize the role of system actors, determining whether and how certain processes have dominant influence for the maintenance of the life cycle activity and/or are more fragile than others, if they require more inputs to deliver the same output flow or are crucial for the system efficiency, the information transfer and the resilience after an occurred shock. We aim here at illustrating a novel method to assess the importance of actors in life cycle production activities. An eMergy calculation software (SCALE) is used to translate LCI datasets in eMergy-LCI matrix systems where flows are converted in one unique biophysical currency (solar emjoule). This allows generating network graphs, where functionality of nodes (LCI processes) can be then explored by means of Graph Theory. Several degree and centrality measures are calculated on the implemented eMergy-LCI graphs of two real LCI case studies, illustrating relative network score trends by process. Results show that a limited number of background processes have mostly a central role in maintaining current targets of system functionality, i.e. the consumption of electricity, some transportation means and the extraction and production of a few abiotic resources. Interestingly, the two analysed systems display similar occurrences (in terms of network performance index trends), suggesting that the common use of the ecoinvent (EIv2.2) database can be of advantage but also a limiting condition. In effect, the analysed systems are not huge if compared to social networks such as Facebook, but they exhibit characteristics of complex networks, and are easy to handle with the tools we have applied. Moreover, EIv2.2 is not sufficiently flexible to allow appropriate resilience analysis, because the functional unit relies on a pre-fixed structure of upstream paths that cannot be modified *a priori*. As shown in the present contribution, using the new EIv3.0 can open insightful ways to detect central roles of actors, because its consequential structure helps determining whether certain shocks imposed to the system (assuming these as policy decisions) may have effect on the resilience and efficiency of the overall life cycle activity.

WEPC06
Environmental impacts at the urban scale – application of different analytical environmental management tools
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CESAM; D. Lemos, University of Aveiro; X. Gabarrell Durany, Universitat Autònoma de Barcelona / Chemical Engineering Department Institut de Ciència i Tecnologia Ambientals; L. Arroja, University of Aveiro
The objective of this study is to apply three analytical tools to an urban scale in order to compare the outcomes of each one. The tools are as follows: life cycle assessment (LCA), environmentally extended input-output analysis (EEIOA) and material flow accounting (MFA). The urban territory studied was the municipality of Aveiro, which is located in the central part of Portugal. A consumption perspective was adopted in the three tools as they refer to the activities required by the households living in Aveiro. The functional unit was the consumption of products and services per citizen and day. In the MFA, city-specific data were collected for some inputs and outputs. These data were complemented with data that were estimated from downscaling of national data. The LCA study adopted the MFA flows as foreground inventory data. Most of the background inventory data were taken from the Ecoinvent database. The impact assessment method used was the ReCiPe 2008 for the impact categories of climate change (CC) and fossil fuel depletion (FFD). The EEIOA relies on the Portuguese economic input-output table, on greenhouse gas emission and fossil fuel consumption intensities per sector in Portugal, and on national household expenditure data downscaled to the urban level. The environmental impacts addressed were CC and FFD, as in LCA. The results obtained with LCA were a total CC impact of 27.3 kg CO_2-eq./cap/day and a total FFD impact of 7.9 koe/cap/day, while the results obtained with EEIOA were 25.8 kg CO_2-eq./cap/day for CC and 7.3 koe/cap/day for FFD. Although the total results obtained with LCA and EEIOA are similar, the individual impacts of each sector or product type are different. Most of the disparities may be explained by methodological differences, mainly related with system boundaries (the products included are not exactly the same due to lack of data), use of different impact data on supply chains, and use of monetary units in EEIOA. LCA allowed the identification of impacts on a product level much more detailed than in EEIOA and, therefore, is considered a better approach when the objective is to identify hot spots for policy support. However, EEIOA was much more simple to develop and less time consuming, which is a great advantage of this tool. Hence, a combination of both tools can probably be the best choice as it allows a comparison of results. MFA has proved to be a useful tool to establish the foreground inventory data required in the LCA study.

Teaching and communicating sustainability – paving the way to a common understanding and meaningful actions (PC)

WEPC07
Communication of sustainability in tourism: examples for Swiss ski tourism, cruises and a public festival
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Tourism is an emotional topic since most of us treasure those precious getaways. Tourist activities are often highly significant in terms of carbon emissions, both from the perspective of a carbon footprint of a single person as well as from a broader sector perspective. These preconditions make carbon footprints of tourist activities suitable for communication. In this work we show three case studies on touristic activities and outline how the results were communicated to the public as well as used for awareness rising. Carbon footprints of tourism activities can be challenging to conduct, for instance because of incomplete or lacking data. Another challenge arises when the outcome is intended for communication. In this work we present the following cases: a cruise calculator, a study on ski tourism in Switzerland and the carbon footprint of the Zürifäscht, a public festival. Nowadays holidays on cruise ships are becoming increasingly popular. The cruise calculator provides a tool for individuals and travel agencies to quantify the impact of this energy intense way of traveling. For the development of the calculator there were contrasting objectives: While on the one hand the intention was a representation which is as precise as possible, on the other hand the tool needs to be easy to understand and use, taking into account different educational backgrounds and intended uses. The second case shows how for Swiss sky tourism different presentation of data changes the conclusions drawn by the beholder, thus influencing the message. The last case study presents the carbon footprint of the Zürifäscht, the biggest public festival in Switzerland. It shows how intelligent mobility concepts are a crucial aspect for mitigating negative impacts in terms of climate change. Here communication measures were directed at the general public whilst at the same time providing organisers with valuable insights for future festival planning. This work shows the importance of taking the communication measures into account in the study design and how various communication channels can be used to raise awareness in terms of carbon friendly choices or design of tourist activities.

WEPC08
Sustainable development integration strategies in higher education: Case study of two universities and five colleges in Quebec
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With the financial support from *the ministère de l'Éducation, du Loisir et du Sport du Québec*, two universities and five colleges(1) in Quebec are developing a project toward the integration of sustainable development (SD). In this project, the integration of SD is not only dedicated to teaching but is also expanded to four different levels: 1) professors' support using various activities (conferences, workshops and short lectures). This support is crucial as it inventories best practices, in addition to a personalised support. In terms of 2) programs integrations, SD integration is seen from a professional perspective when students will have to apply their knowledge in a day to day basis during their work. A good example is the engineering and business disciplines. At the third level (the institutional one), some institutions have adopted various politics and actions to foster SD in the curriculum and regarding their overall missions of research, community outreach and operations. Finally, the forth level consists of a regional collaboration between the 7 presented institutions, which is a unique characteristic of this project. This work is advocating an integrated strategy of variable geometry, as in one hand, it recognises the expertise of professors in their field while on the other hand; it implements structuring actions throughout programs and establishments. As an example, a two-day workshop is offered on a regular basis gathering teachers from various disciplines and brainstorming strategies beyond "constraining" administrative boundaries. These practices helped in building more recently a critical mass of practitioners in the field and launched a community of practice (working group) in integrating SD in higher education. Preliminary results show that the integration of SD into student education is not only accomplished through pedagogical activities, peri-academic and extracurricular activities but is also embodied in the overall student experience on campus. SD's main stake is to grow into an institutional culture in which teaching, research and management activities mutually reinforce each other. (1) Université de Sherbrooke, Bishop's University, Cégep de Sherbrooke, Cégep de Drummondville, Cégep de Granby – Haute-Yamaska, Champlain Regional College Lennoxville and Cégep de

Victoriaville (Cégep denotes for College)

WEPC09
Communicating and teaching sustainability: Using LCA as a metrics in advancing Green Chemistry
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The concept of Green Chemistry was coined in the 1990s and can be briefly defined as applying the pollution prevention approach to chemistry. LCA is a technique to assess environmental impacts associated with all the stages of a product's life from-cradle-to-grave. If this method is applied in the form of a gate-to-gate LCA it provides the environmental profile, or footprint, of a chemical or material composed of multiple chemicals. A criticism to the 12 green chemistry principles as a tool is their qualitative nature. Life Cycle Assessment is applied in three cases to demonstrate how it can be used as a metrics to quantify the results achieved on the basis of the green chemistry principles: production of maleic anhydride by two synthesis ways, production of hydrogen from vegetable oils and production of emulsion-templated porous materials from Kraft black liquor. Using these three examples, this paper shows how LCA can help evaluating the 12 principles in concrete cases of advancing green chemistry research towards the sustainability of chemicals. Emphasis is made to show the potential for using LCA as a metrics in scaling up this research. The three cases presented have been used systematically in communicating to chemists who in general are well aware of the GC principles but not of the benefits of using LCA for communicating and innovated towards sustainability. The three relatively simple examples put into evidence that using LCA is useful to advance Green Chemistry research since it facilitates the choice of the most environmentally sustainable path forward and provides a consistent basis for a professional communication about relevant sustainability characteristics like carbon footprint. These examples have also been used successfully in teaching chemistry students about sustainability assessments as an extension to the conventional courses on environmental management and risk assessment. It could be observed that those students accustomed to the green chemistry approach were more interested in listening to an LCA lecture if they had been motivated about the usefulness of LCA for moving towards sustainability when they had previously heard about the cases mentioned above that correspond to the daily challenges they face in their projects and internships in chemistry labs. **\n**

WEPC10
The DPSIR approach applied to marine eutrophication in LCIA as a learning tool
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The Drivers-Pressures-State-Impacts-Responses (DPSIR) is an adaptive environmental management approach that integrates environmental, social and economic aspects into a common framework. It deals with the Drivers (D) that generate the Pressures (P) e.g. from human interventions, that modify the State (S) of the ecosystem, causing the Impacts (I) on these, and contributing to the management strategies and Responses (R). The latter are designed to modify the drivers, minimise the pressures and restore the state of the receiving ecosystem. In our opinion the DPSIR provides a good conceptual understanding that is well suited for sustainability teaching and communication purposes. Life Cycle Impact Assessment (LCIA) indicators aim at modelling the P-S-I parts and provide a good background for understanding D and R. As an example, the DPSIR approach was applied to the LCIA indicator marine eutrophication. The goal is to promote an educational example of environmental impacts assessment through science-based tools to predict the impacts, communicate knowledge and support decisions. The example builds on the (D) high demand for fixation of reactive nitrogen that supports several socio-economic secondary drivers. The nitrogen exported to marine coastal ecosystems (P), after point and nonpoint source emissions, promote changes in the environmental conditions (S) such as low dissolved oxygen levels that cause the (I) effects on biota. These, stimulate society into designing actions (R) to modify D, reduce P, and restore S. Concrete responses can be technical (e.g. increasing sewage treatment coverage), regulatory (e.g. EC Nitrate Directive) or guidance (e.g. fertilisers formulation or best practices for application). These should consider six basic tenets for environmental management: environmentally sustainable, technologically feasible, economically viable, socially desirable, legally permissible, and administratively achievable. Specific LCIA indicators may provide preliminary information to support a precautionary approach to act earlier on D-P and contribute to sustainability. Impacts assessment and response design ultimately benefit from spatial differentiation in the results. DPSIR based on LCIA seems a useful tool to improve communication and learning, as it bridges science and management while promoting the basic elements of sustainable development in a practical educational application. Other LCIA indicators can also be adapted to fit similar purposes.

Bioaccumulation processes and mechanisms: Implications for experimental assessments and modelling (PC)

WEPC13
Interrelationship of bioaccumulation metrics (BCF, BAF, BMF, and TMF) and how they may be incorporated into a screening-level probabilistic risk assessment.
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The potential of a chemical to accumulate in living organisms (bioconcentration) and increase in concentration with increasing trophic level within a food web (biomagnification) are important considerations for assessing bioaccumulation and ecological risk. Measures used to assess bioaccumulation take into consideration that pathways of exposure may occur through various sources (i.e., water, sediment, diet, and air). For aquatic organisms, bioconcentration factors (BCF) describe the uptake and accumulation of chemicals from water only. Bioaccumulation factors (BAF) describe uptake and accumulation from all sources relative to the amount of chemical stored in the water compartment. Biomagnification factors (BMF) describe the increase in concentration of chemicals in organisms that are separated by a single trophic level step on a food chain. Similarly, trophic magnification factors (TMF) describe the increase in concentration of chemicals in organisms that occupy successively higher trophic levels within a food web. Regulatory screening and assessment criteria used to identify potential bioaccumulative substances are typically based on laboratory measurements of BCF. This presentation will show how these metrics of bioaccumulation are algebraically interrelated and may be expressed in terms of a "***bioaccumulation equation***", which shows that concentration in an organism is a function of the BCF, the exposure concentration, the BMF (or TMF), and the trophic level occupied by the organism. The bioaccumulation equation will be used to demonstrate (1) that BCF is not a valid measure of biomagnification; (2) that BCF alone is not a good indicator of bioaccumulation or for identifying a bioaccumulative substance of concern; and (3) how BAF, BMF, and TMF may be incorporated into the regulatory screening and assessment process. These interrelationships will also be applied to select contaminants to demonstrate how margin of safety considerations maybe addressed by incorporating probabilistic risk assessment methods.

WEPC14
A dynamic multi-compartment fish bioaccumulation model with a focus on dietary chemical absorption
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Dietary absorption is the most important pathway for uptake of highly hydrophobic chemicals by fish. To mechanistically describe this process, we developed a fugacity-based dynamic model that accounts for different uptake and elimination processes, providing insight into evolution of contaminant concentration. Our new dynamic, fugacity-based multi-compartment fish bioaccumulation model describes the fish gastrointestinal tract (GIT) as two discrete compartments. We illustrate the model by applying it to describe chemical uptake and clearance in rainbow trout for a consistent set of dietary testing data that used a single gavage dose administered in gelatin capsules. The multi-compartment fish model is initially used to describe a dataset of chemicals including seventeen halogenated benzenes and biphenyls, seven dioxins, and sixteen diphenyl ethers. The model explains 72% of the variation in observed concentrations (n=189, p< 0.01) of these substances, without a large apparent bias. The dataset also includes experimental data (n=26) for six polycyclic aromatic hydrocarbons (PAHs) and the model over-predicts the concentrations by approximately one order of magnitude. The unfavorable agreement between the measured and modeled values for the PAHs can be ameliorated by estimating and including rates of degradation assumed to occur in the GIT. Our results corroborate previous studies that biotransformation of PAHs in the GIT plays an important role in the absorption process.

WEPC15
Mechanistic PBTK modelling for in vitro up-scaling
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There has been much recent work on developing and validating in vitro tests of the metabolic clearance of chemicals using fish liver enzymes. To make these measurements useful in the regulatory context models have been employed for up-scaling to whole body clearance values and bioconcentration factors. These models are typically steady-state, one compartment pharmacokinetic models with chemical partitioning based on regressions with log K_{OW}. We have developed a detailed multicompartment Physiologically-Based ToxicoKinetic (PBTK) model for fish based on mechanistic uptake, distribution, and excretion processes for

neutral compounds. One of the advantages of the model is a realistically defined metabolism in the intracellular space of the liver, and a fully defined blood flow system in the fish. Most experimental measurements performed on fish collect whole body data such as bioconcentration factors. This data has been used in a mass balance approach to calculate whole-body metabolic clearance rates. Using kinetic parameters from in vitro experiments in the intracellular compartment of the model and running a simulation that describes the uptake, distribution, and excretion of a chemical will allow a whole body metabolic clearance to be estimated. From the whole body metabolic clearance data inverse modelling by stepwise adjustment of the metabolic clearance rate in the liver allow a corresponding downscaling of the whole body data to kinetic parameters comparable to the in vitro data. This modelling provides a method for comparing the different kinds of metabolic data, which is useful for metabolic clearance studies in the environment as well as for pharmacology. In a case study with several compounds, where we found literature data for both in vitro and whole body metabolic clearance rates. Up-scaling and inverse modelling simulations are performed and the results and conclusions presented

WEPC16
Evaluation of non-animal methods as alternatives to in vivo fish bioaccumulation studies
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The potential bioaccumulation of a compound is usually expressed by the bioconcentration factor (BCF), which is measured in whole fish according to the Organisation for Economic Cooperation and Development (OECD) Guideline 305.BCFs can also be determined through alternative methods to the OECD protocol, including non-animal models such as *in silico* models and *in vitro* test systems.Although *in vitro* test systems have not yet been standardised, they can provide data on the role of biotransformation (metabolism) in the bioaccumulation of chemicals. Metabolic activity is usually determined by the intrinsic hepatic clearance rate that can be used to calculate the whole body biotransformation rate (Kmet). The aim of this study was to evaluate non-animal methods as a potential surrogate, or complement, to *in vivo* OECD studies for the assessment of chemical bioaccumulation for a set of compounds. Non-animal methods employed included (1) an *in silico* model of the (maximal) log BCF-log Kow relationship, (2) an *in vitro* assay using freshly isolated hepatocytes from rainbow trout to calculate Kmet and (3) the Arnot and Gobas model for the prediction of BCF from the *in vitro* Kmet data generated in the *in vitro* assay. Nine chemicals were selected for *in vitro* testing based on their *in vivo* BCF values, which were compiled from the Environment Canada Domestic Substance List (DSL) and non-DSL Environment Canada databases. From the analysis of multiple log BCF data for such compounds, the relationships between BCF and hydrophobicity was derived. Generally, the Arnot and Gobas model underestimated the BCF for compounds with log Kow < 4.5; however, it made better predictions for chemicals with log Kow > 4.5 than the log Kow-based model. This finding is in agreement with previous studies reporting that metabolism may have a large influence on the bioaccumulation for hydrophobic compounds. This study provides the foundation for potential evaluation and development of alternative methods to assess the bioaccumulation of chemicals. The *in vitro* clearance assay, in combination with the Arnot and Gobas model, represents a potential surrogate to *in vivo* studies with respect to the traditional log Kow-based models. However, more experimental effort is still needed to improve non-animal methods for bioaccumulation assessment.

WEPC17
Predicting bioconcentration of fragrance ingredients by an in vitro-in vivo extrapolation model based on biotransformation rates in trout liver S9 fractions
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Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals. In general, this requires the determination of a fish bioconcentration factor (BCF). Relatively little *in vivo* BCF data are available for fragrance ingredients. Thus, predictive models which are mainly based on hydrophobicity (i.e. log K_{ow}) are commonly used to estimate their BCFs. Since biotransformation can reduce the extent of accumulation, predictive models using estimated biotransformation rates were developed. *In vitro* systems measuring fish metabolism of test compounds to refine BCF computer model estimates have been proposed as alternative methods that may be used to substitute for *in vivo* BCF determination. The goal of this study was to compare BCF predictions based on measured *in vitro* biotransformation rates in trout liver S9 fractions to BCF estimations based on predicted biotransformation rates and predictions assuming no biotransformation for nine fragrance ingredients (log K_{ow} 4.0 to 5.8) with known *in vivo* BCFs. Metabolic stability was determined by monitoring the disappearance of the parent molecule [1] using GC-MS analysis. The biotransformation rates then served as input for a recently refined *in vitro-in vivo* extrapolation model to predict BCF values [2]. For the majority of molecules tested, we observed moderate to rapid enzymatic turnover by trout liver S9 fractions. No significant enzymatic turnover was found with Musk xylene, while slow turnover was observed for three chemicals. For fragrance ingredients with relatively low log K_{ow} values, BCF predictions are unaffected by metabolism [2]. For the more lipophilic substances like Isolongifolanone, Opalal, Iso E Super and Methyl cedryl ketone, a better BCF prediction was obtaining when accounting for metabolism. These BCF estimates obtained with the refined model closely reflected *in vivo* BCFs. Most importantly, these estimations did not lead to underprediction of the BCFs, but avoided the strong overpredictions due to classical log K_{ow}-based models. *In vitro* S9 metabolism data in combination with the refined *in vitro-in vivo* extrapolation model are a valuable tool to assess bioaccumulation potential as part of a weight of evidence approach. References*:* [1] Johanning, K. et al. 2012. Current Protocols in Toxicology 14.10.1-14.10.28. [2] Nichols, J. et al. 2013. Environ Toxicol Chem 32 (7), 1611-22.

WEPC18
A benchmarking approach to determine biotransformation rate constants for organic chemicals in fish from in-vivo experiments
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We present an *in-vivo* fish experimental test protocol that exploits internal chemical benchmarking to measure absorption efficiency and biotransformation rate constants for organic chemicals. We tested twelve substances that are alkyl-substituted mono- and di-aromatics and cyclic siloxanes with log K_{ow}-values ranging from 5 to 10. The chemicals were selected because they have a wide range of biotransformation rate constants based on the BCFBAF QSAR model included in the EPI Suite software. Fish were given a single dose of feed contaminated with the test substances and the benchmarking chemicals simultaneously, followed by a depuration phase that lasted for 6 weeks. The contaminated feed also included five benchmarking chemicals: ß-hexachlorocyclohexane, hexachlorobenzene, PCB 52, PCB 153 and PCB 209. Decabromodiphenyl ethane was added to the fish feed once a week as a non-absorbable benchmark for estimation of feces collection efficiency. Experiments were conducted in two fish tanks containing 40L of aerated water, each containing ten rainbow trout weighing about 80 g each. Feces were collected from the bottom of the tank every day by siphoning. The slurry was filtered through a glass-fiber filter, dried, weighed, extracted, cleaned-up, and analyzed by GC/LRMS. Every week, two fish from each tank were sampled and homogenized. Aliquots of the homogenate were extracted, cleaned-up, and analyzed by GC/LRMS. Benchmarking against decabromodiphenyl ethane was used to correct feces collection efficiencies to 100%, but concentrations in feces were low because absorption efficiencies for all test compounds were close to 100%. The PCBs and hexachlorobenzene were used as benchmarks to estimate growth dilution of the initial dose of chemical during the experiment, and ß-hexachlorocyclohexane provided a benchmark to estimate gill elimination. Biotransformation rate constants could be estimated for seven of the test chemicals from benchmarked depuration kinetics. The measured values correspond well to estimates from the BCFBAF QSAR in all but one case.

Research on communication and communication of research – pinpointing the best practice to improve our outreach (PC)

WEPC19
'Evaludation': Merging validation and evaluation of ecological models
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Ecological effect models have long been identified as useful tools to extrapolate experimental findings to more realistic conditions, such as larger temporal or spatial scales, or higher levels of biological organization. However, confusion about model validation is a major challenge and obstacle in using ecological models for decision support. Unclear terminology is one of the main hurdles to a good understanding of what model validation is, how it works, and what it can deliver. In a literature review of terminology related to this issue, we identified 'validation' as a catch-all term, which is thus useless for any practical purpose. We therefore introduce the term 'evaludation', a fusion of 'evaluation' and 'validation', to describe the entire process of assessing a model's quality and reliability. Considering the iterative nature of the modelling cycle, we identified six essential elements of evaludation: (i) 'data evaluation' for scrutinising the quality of numerical and qualitative data used for model development and testing; (ii) 'conceptual model evaluation' for

examining the simplifying assumptions underlying a model's design; (iii) 'implementation verification' for testing a model's implementation in equations and as a computer program; (iv) 'model output verification' for comparing model output to data and patterns that guided model design and were possibly used for calibration; (v) 'model analysis' for exploring a model's sensitivity to changes in parameters and process formulations to ensure that the mechanistic basis of main behaviours of the model has been well understood; and (vi) 'model output corroboration' for comparing model output to new data and patterns that were not used for model development and parameterisation. We believe that the proposed set of terms and its relation to the modelling cycle can help to make quality assessments of ecological models more comprehensive and transparent as well as help to overcome terminological hurdles. Currently, the terminology presented here is being adopted as the basis of the standard documentation format TRACE, which aims at providing supplementary evidence that a model was thoughtfully designed, correctly implemented, thoroughly tested, well understood, and appropriately used for its intended purpose.

WEPC20

The tribes from the chemistry planet: how they communicate to each other?

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Chemistry is a really fragmented research field. This presentation shows how some disciciplines such as chemistry have been splitted in various very specialized fields, but environmental questions need to re-unify them...Are they speaking the same language between us?

WEPC21

Consumers' right to know about risks when using Personal Care Products in Europe and South Africa

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Legal regulations for risk communication should bridge the gap between (eco-)toxicological data generated by experts and risk perception by the public. If consumers benefit from their "right to know" by an efficient risk communication, they might alter their behaviours which may add to reduced health problems and reduced environmental contamination. We analyzed how (eco-)toxicological data on hazardous ingredients in personal care products are communicated to the public in a developed region, like the EU, compared to a developing country, like South Africa. According to our results, risk communication of personal care products is neither satisfactory in Europe, nor in South Africa. Most personal care products contain hazardous ingredients classified according to the CLP-Regulation, and current legislation in the European Union and South Africa does not require these to be labelled as hazardous products. Instead, ingredients must only be listed on containers to inform consumers of potential hazards. Consumers are expected to understand these ingredient lists and to conduct their personal risk assessment. For understanding ingredient lists consumers need high literacy levels, a scientific background, knowledge of labels as a risk communication tool, and the means to comprehend this information. Furthermore, the use context, formal education and cultural factors of the consumers must be considered when developing risk communication mechanisms. As the process of communicating risks is not static, nor are there universally agreed upon approaches apart from GHS, regular surveys are needed to find out in what way the users comprehend the information and whether the risk communication designs would need adjustments. All these factors illustrate the complexity in communicating risks to a lay population and that consumers are currently not provided with information that promotes their right-to-know. We propose several risk mitigation measures which would not only reduce the number of people suffering form negative health effects, but they also would help save costs for the health care systems and environmental remediation.

WEPC22

Emerging pollutants from animal husbandry - Risk assessment and communication

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The growing consumption of antibiotics is not restricted to human beings, this phenomenon is also observed in the intensive animal husbandry. The risk of accumulation of pharmaceutical residues in the water cycle is therefore of increasing concern for environmental scientists. The use of slurry as a fertilizer in the agriculture increases the risk of antibiotics entering the water cycle through this pathway and is getting the attention from the wide public. Due to its high uncertainty this sensitive subject is especially emotionalizing and there is a strong demand for clarification and "translation" of the scientific results to the public. In this context the challenging risk assessment is subject to various research studies also in the RiskAGuA project (sponsored by the German BMBF). In this project additionally best practices in risk communication are investigated and recommendations for efficient communication of scientific results are elaborated. On this basis related policy recommendations are prepared and their consequences are evaluated in an integrated way in order to demonstrate the efficiency of suggested measures.

WEPC23

Evaluation of two methods of scientific outreach for adolescents in Barcelona (Catalonia, Spain)

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It is often recognized that science is not well communicated to, neither understood by the general public. The problem is due to two major reasons: a) Scientifics spread their work and results in a complex way, and using a jargon, aimed to colleagues with a similar background, and b) there is a common lack of interest of the population regarding Environmental Sciences. Nowadays, diffusion of science is gaining importance in all spheres and communicational skills are abilities increasingly valuable in scientists. Regarding the second driving force, it is necessary to adapt the methodology of scientific divulgation to the age, social condition and initial interest of the public. We focused especially in the initial educational stages, childhood and adolescence, as it is still a very accessible audience open to unfamiliar topics. The objective of the present work is to encourage young students to be not only interested, but captivated by science through active participation, and in addition, to transmit this interest to their relatives. The Spanish National Research Council (CSIC) has developed two programs within schools and high schools to get science closer to students between 12 and 18 years old. In each of them we evaluated two different methods for scientific divulgation addressed to adolescents. The first one was tested during the Catalonian program *CSIC at the Classroom*, in which scholars and PhD students visited the schools involved in the program, and run talks and activities to bring science to the own surrounding of the teenagers. The second method was used during the *CSIC Science Week* program, and comprised the active participation of students in several workshops in different Research Institutes from CSIC, including IQOG and IDAEA. In both experiences the contents were our real study field; environmental pollution and distribution of organic pollutants over the planet. Concept acquisition and productivity of students between 16-18 years old were higher when visiting the research center than at the classroom; whereas students between 12-15 years old showed better learning results in their own classroom. Several reasons of the differentiation will be assessed and results will corroborate the importance of fitting the spreading methodology to the age range in order to communicate science as a friendly and important topic, increasing the future spread of Environmental Sciences in our society instead of an unknown issue exclusive for Scientifics.

Keyword Index

SETAC Europe 24th Annual Meeting

Author Index

SETAC Europe 24th Annual Meeting

C

SETAC Europe 24th Annual Meeting

D

E

F

G

SETAC Europe 24th Annual Meeting

H

I

J

SETAC Europe 24th Annual Meeting

K

L

SETAC Europe 24th Annual Meeting

M

N

O

P

Q

R

S

T

U

SETAC Europe 24th Annual Meeting

SETAC Europe 24th Annual Meeting

Z

The Society of Environmental Toxicology and Chemistry (SETAC) is a not-for-profit, global professional organisation comprised of some 5500 individual members and institutions dedicated to the study, analysis and solution of environmental problems, the management and regulation of natural resources, research and development, and environmental education.
SETAC Europe is one of five Geographic Units of the global Society, established to promote and undertake activities of SETAC in Europe, and to support the activities of SETAC in the Middle East and Russia. The Society is dedicated to the use of multidisciplinary approaches to examine the impacts of stressors, chemicals and technology on the environment. We also provide an open forum for scientists and institutions engaged in the study of environmental problems, management and regulation of natural resources, education, research and development, and manufacturing. SETAC Europe is incorporated in Belgium as a not-for-profit organisation. The Society is governed according to its articles of association and by-laws. SETAC Europe maintains its administrative office in Brussels, Belgium.

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