RIVM Report 860706002/2007

Exposure and ecological effects of toxic mixtures at field-relevant concentrations

Model validation and integration of the SSEO programme

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This study has been funded by a research grant from the Netherlands Organisation for Scientific Research (NWO); it is part of the Stimulation Programme System-Oriented Ecotoxicological Research, an initiative of the Ministries of LNV, V&W, OC&W and VROM.

ISBN: 978-90-6960-181-6

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Abstract

Exposure and ecological effects of toxic mixtures at field-relevant concentrations

Local environmental conditions need be taken into account to assess the impacts of diffuse environmental pollution on ecosystems. This was shown in an evaluation of the scientific results of the Dutch Stimulation Programme System-Oriented Ecotoxicological Research, led by RIVM.

Effects of diffuse pollution on the environment were studied at three contaminated areas in the Netherlands: the flood plains of a large lowland river (Waal), a tidal area (Biesbosch) and a peat soil area (near Vinkeveen). Diffuse pollution is present in these areas. Type and magnitudes of effects were determined and analysed.

The integrated results analysis showed that effects ranged from absent or low, to clearly visible and large. The magnitude of effects depended on the local composition of the mixture, the local characteristics of the soil, the water or the sediment, and the sensitivity of the local micro-organisms, plants and animals being exposed. The combination of these three characteristics influenced the type and magnitude of impacts.

The measurement methods and the modelling analyses used in the research programme appeared to be useful for managing the risks of diffuse site contamination. Application of these methods and analyses is useful for the Dutch situation, given the large areas with diffuse contamination. Sanitation of large, diffusely contaminated areas is not a practicable solution. RIVM recommends the development of a toolbox for ecological risk assessment. Application of such a toolbox will lead to a better assessment of local risks, and the assessment will be based more on ecological approaches. Eventually, this will result in linkage between substance-oriented policies and the management of contaminated sites.

Trefwoorden / Key words:
Diffuse pollution, ecosystem responses, risk assessment, risk management

Rapport in het kort

Blootstelling aan en ecologische effecten van toxische mengsels bij veldrelevante verontreinigingsituaties

Om de effecten te voorspellen van giftige stoffen die zich diffuus in het milieu verspreiden is het nodig om de lokale milieucondities in kaart te brengen. Dit blijkt uit een evaluatie van resultaten uit het Nederlandse Stimuleringsprogramma Systeemgericht Ecotoxicologisch Onderzoek (SSEO) die uitgevoerd is onder leiding van het RIVM.

De afgelopen zes jaar zijn op drie verontreinigde locaties in Nederland de effecten onderzocht van giftige stoffen op milieu, planten en dieren. De locaties betroffen de uitwaarden van een grote rivier (de Waal), een getijdegebied (de Biesbosch) en een veenweidegebied (nabij Vinkeveen). Op deze plekken hebben zich giftige stoffen verspreid over de omgeving. Van deze diffuse verontreinigingen werden de omvang en effecten gemeten en geanalyseerd.

Uit het onderzoek blijkt dat de effecten varieerden tussen niet-waarneembaar of zeer gering tot waarneembaar en groot. De grootte van de effecten hing af van de aanwezige stoffen en hun concentraties, de eigenschappen van bodem, water of sediment op de locatie, en de gevoeligheid van planten en dieren die werden blootgesteld aan de stoffen. Dit maakt duidelijk dat milieucondities voor een deel de effecten van de stoffenmengsels bepalen.

De meetmethoden en modelanalyses van het SSEO-programma blijken bruikbaar voor het beheersen van lokale risico's van verontreinigingen. Voor Nederland is het heel belangrijk om deze instrumenten op grotere schaal toe te passen gezien de vele diffuus verontreinigde locaties. Saneren is op die plekken geen oplossing. Om de risico's van deze verontreinigingen te beheren adviseert het RIVM een risicotoolbox te ontwikkelen. Toepassing daarvan is nodig voor een betere op ecologie gebaseerde effectbepaling. Dit kan uiteindelijk leiden tot een koppeling tussen stoffenbeleid en gebiedsbeheer.

Trefwoorden / Key words:
Diffuse verontreiniging, ecosysteemeffecten, risicobeoordeling, risicobeheer

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Preface

This report describes the results of data integration, model validation and scientific integration of findings at the completion of a major national research programme in the Netherlands into the ecotoxicological field effects of mixtures of contaminants at realistic ambient concentration: the NWO/SSEO programme (Stimulation Programme System-Oriented Ecotoxicological Research). This programme was commissioned by the Ministry of Housing, Spatial Planning and the Environment (in Dutch: VROM), the Ministry of Agriculture, Nature Management and Food Safety (in Dutch: LNV) and the Ministry of Transport, Public Works and Water Management (in Dutch: V&W). It was carried out under the auspices of the Netherlands Organisation for Scientific Research (NWO), by means of a Steering Committee (chaired by Prof. Dr. K. Verhoeff) and a Programme Committee (chaired by Prof. Dr. H.J.P. Eijsackers). This report is based on more than 100 original scientific studies, of which the publication in the form of reports and journal publications has been finished or is in progress. The reader should refer to those original scientific reports for details.

The report summarizes the results of a set of validation studies of a selected number of ecotoxicological models that are used by the governmental research institutes Alterra, RIZA and RIVM to support the environmental policy for and risk management of toxic compounds. The overview of results of these summaries is preceded by information on the objectives of the programme, characteristics of the study sites, and the approaches followed in this study. The overview is followed by an initial integration and interpretation step. This links the scientific findings explicitly to current policies and ongoing policy developments.

Preceding reports from the starting phase of the programme, such as those published by RMNO and NLRO[1], established the policy need for ecosystem-oriented ecotoxicological research. Such reports were the basis for establishing the SSEO research programme. Regarding integration and implementation of research findings of the programme, a previous report (RIVM report no. 860706001) presented an inventory of the toxic compounds being studied, the model parameters being measured and the models that are relevant for ecosystem-oriented ecotoxicological research and the model validation studies. The validation and integration studies as presented in the present report (RIVM report 860706002) were commissioned by the Dutch research programme “SSEO”. The work on this report was coordinated by a research consortium comprising Alterra, RIVM and Radboud University Nijmegen, managed by the Laboratory for Ecological Risk Assessment of RIVM. SSEO provided a research grant to this consortium in 2002 to execute a set of model validation studies. This consortium has grown over time to include the researchers, universities and institutes that actually wrote this report with the aim of providing a bird’s eye view of the results of the SSEO programme. Where relevant, data from complementary studies have been used to reinforce and further clarify findings of the studies.

For further information, the reader can refer to presentations made at a Special Symposium related to SSEO at the conference of SETAC-Europe that was held in The Hague in May 2006. Additional related publications from the SSEO programme consist of yearly progress reports and project reports (compiled by NWO), and an array of research papers and PhD theses, part of which were prepared by the research team running the integration and validation project. Moreover, a special issue of the journal *Science of the Total Environment* is being prepared, and various overview presentations in Dutch magazines (such as the journals *Bodem* and *Milieu*) have been published. Furthermore, the SSEO Steering Committee and Programme Committee have elaborated on the scientific results with publications that focus on the policy implications of the SSEO programme results. The editors of the present report (LP and MV) are indebted to all the researchers who contributed by providing data, helping with data compilation, providing draft publications, chapter texts and so forth. Without their help, neither the data integration nor the validation and integration studies could have been performed! The SSEO programme committee (HE) gratefully acknowledges all these efforts, but especially the untiring dedication of the editors to achieve this impressive result. **HE, MV, LP**

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[1] RMNO is the Dutch Advisory Council for Research on Spatial Planning, Nature and the Environment; NLRO is the former Dutch Advisory Council for Agricultural Research

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Executive Summary

The ecological effects of diffuse pollution involving mixtures of toxic compounds cannot be determined from exceedances of national environmental quality criteria for toxic compounds. Local conditions strongly influence the effects of such pollution. Applying an ecology-based, site-oriented approach in risk assessment allows for better recognition of many effects of diffuse contamination. It implies a focus on ecological criteria and on options for management with the aim of approaching a Good Ecological Status, rather than a focus solely on the exceedance of generic quality criteria for toxic compounds. Combining both approaches can be of help in linking pollution prevention policies with policies focusing on the environmental compartments of water, sediment and soil, and to those focusing on the protection of biological species and ecosystems. This was the conclusion of a broad evaluation of the scientific results of the Dutch Stimulation Programme System-Oriented Ecotoxicological Research[2].

Diffuse contamination is present at the three research sites of SSEO, and is also present elsewhere. Diffuse contamination is defined as the exceedance of the regulatory Target Value for toxic compounds in an environmental compartment. Inventories of such exceedances indicate that, in a regulatory sense, “non-clean” environmental compartments exist in large numbers, volumes and areas. This implies the need for a policy choice to handle those sites. From the viewpoint of authorities responsible for diffusely contaminated sites, there is a clear regulatory and societal trigger to investigate the implications of diffuse contamination for ecosystems. In societal terms, exceedances of quality criteria are often interpreted in terms of the presence of unacceptable adverse effects, while the exceedances conceptually imply the presence of unacceptable risks (increased probability of unacceptable effects). So far, few if any approaches have been adopted to assist site managers.

This report describes the results of data integration, model validation and scientific integration of findings at the conclusion of a major national research programme in the Netherlands into the ecotoxicological field effects of mixtures of contaminants at realistic ambient concentrations; the NWO/SSEO programme (Stimulation Programme System-Oriented Ecotoxicological Research). This programme was commissioned by the Ministry of Housing, Spatial Planning and the Environment (in Dutch: VROM), the Ministry of Agriculture, Nature Management and Food Safety (in Dutch: LNV) and the Ministry of Transport, Public Works and Water Management (in Dutch: V&W). It was carried out under the auspices of the Netherlands Organization for Scientific Research (NWO).

The effects of diffuse and serious contamination in the environment were studied in the SSEO research programme. The studies in the programme focused on three Dutch areas with known diffuse pollution gradients: the flood plains of a large lowland river (de Waal), a freshwater tidal area (de Biesbosch) and a peat-soil area (near Vinkeveen). The sites are characterized by the long-term presence of the toxic compounds in the soil and sediment and by contamination gradients. The flood plains and the tidal area were contaminated due to human activities that influenced the water bodies. The peat soil was contaminated due to deposition of household waste a few hundreds of years ago. The peat soil site is relatively stable as compared to the two other sites, where flooding events imply relatively high system dynamics.

Many researchers (PhD students and postdoc researchers) have been working at the three selected locations to study system characteristics and the characteristics of species abundances or species

[2] In Dutch: SSEO, Stimuleringsprogramma Systeemgericht Ecotoxicologisch Onderzoek

assemblages, and to establish whether biotic characteristics are associated with the type and degree of contamination. Both abiotic and biotic parameters were measured; these included local soil pH, data on the type and degree of contamination, assessments of the soluble fraction (hypothetically bioavailable), tissue residue measurements in various field-collected specimens and species abundance counts for various species. The research activities were extended where appropriate with field, semi-field and laboratory tests. To integrate all findings, one project focused on data integration, the validation of various ecotoxicological models and the implications of the research findings for policy. Possible implications were defined for a range of policies, since diffuse contamination is a problem addressed in chemical-oriented policies, compartment-oriented policies (soil, water) and nature policies.

This research has demonstrated a wide range of ecological responses, ranging from little or no effects at high exposures to large effects on valued species or ecosystem characteristics at low exposures. The wide variability is attributed to three factors (each with large variation between sites):

a. The local mixture (composition, concentrations)
b. The local site characteristics that influence exposure through different sorption characteristics and processes like ageing
c. The locally-exposed biota, with varying sensitivities, for which effects are further influenced by biotic interactions at higher levels of biological organization (communities).

Finding effects in diffusely exposed systems may be difficult due to the effects of other stress factors in the natural systems.

From the perspective of national and international regulations, this important finding implies that diffuse pollution is a complex problem, since it is dealt with in various regulations that have both a good chemical status and a Good Ecological Status (or similar concepts, like vital populations of target or non-target species) as final endpoints.

For authorities responsible for diffusely contaminated sites, diffuse pollution remains a complex problem, since they can use environmental quality criteria to classify the sites as clean, slightly contaminated or seriously contaminated, but thereafter have access to only a few or perhaps no accepted methods for local risk management.

In view of the diversity of effects and the underlying causes, the SSEO programme has not resulted in additional evidence to suggest a general change in the processes whereby generic risk assessment is used to derive environmental quality criteria and prevention principles are used to avoid releasing dangerous novel compounds on the market. However, the diversity of the effects suggests that using exceedances of quality criteria to guide risk management is not advisable as sole assessment in cases of diffuse pollution. Such exceedances as yet offer no insight into the kind and magnitude of ecological responses. Generic risk limits are simply not equivalent to quantitative effects, due to the three major factors mentioned above. From the viewpoint of chemical regulation, changes in generic risk assessment could systematically yield criteria that are more stringent or less stringent. Another validation to reach the same status of regulatory acceptance as the current, generic approach (validated in the sense that they most often provide sufficient protection) is needed. This will take some time, because before such novel methods can be adopted, it must be shown that they improve the commonly used evaluation technique.

From the point of view of responsible site authorities, the use of quality criteria does not sufficiently solve the risk management problem of ecosystems already contaminated, since cleaning up sites that are exposed at levels higher than Target Values (or Intervention Values) is not realistic due to their huge numbers and vast volumes. Other methods are needed to support the authorities responsible for the management of contaminated sites. When considering both types of regulation (chemical-oriented versus site- or species/ecosystem-oriented), a possible incompatibility between approaches is suggested. However, this is artefactual. The quality criteria were specifically designed for a limited use (i.e., prevention and detection of unacceptable risks), and it has always been implicitly accepted that further investigation is necessary to describe the ecological consequences of exceedances. Thus, efforts are needed to embed the concept of tiering, explained below, in any set of regulatory frameworks.

The SSEO programme has applied many methods to investigate local exposure levels and the occurrence and magnitude of local effects of diffuse contamination. The methods consist of measurement techniques, modelling approaches, or combinations of both. In some cases, a formalized triad approach has been applied. This approach is based on the concept of Weight of Evidence, by weighting observations from different Lines of Evidences (e.g., modelling, bioassays, field inventories). The methods can be brought together in a site-oriented approach in risk assessment. They can be considered candidate methods to be applied in a tiered system of risk assessment, whereby the first tier consists of evaluation using generic quality criteria (screening-level risk assessment to discriminate sites), and the higher-tier approaches consist of refined, site-specific risk assessments. All tiers universally apply the risk assessment paradigm (i.e. basic consistency between tiers), but only the refined assessment considers more site-specific information in the exposure and effects assessment steps. This yields more refined information on the local risks. Often, according to general principles for tiered systems, the results of refined assessments are less conservative than those of screening-level assessments. Again from the viewpoint of national and international chemical regulation, the more widespread adoption of a tiered system is a logical extension of earlier adoptions of this principle. A higher-tier method would be required, especially for sites with existing diffuse contamination. As a result, there will be three regulatory approaches: prevention, risk management in cases of diffuse pollution, and curation in cases of serious pollution. According to law, in addition, clean-up is mandatory for novel cases of pollution.

A tiered system should not only address the site contamination itself (as based on the ecotoxicological implications of contamination), and *limitations of use* implied by that, but also scenarios for *optimized site management*. The latter should result in the best ecological status that can be reached in the local context. Practical methods are needed to assess not only the current ecological status (like the Good Ecological Status concept) and risks of current site use, but also the ecological perspectives for the development and use of contaminated sites in case changes of site use are planned. Scenario analyses could have great potential in this respect, regarding limitations (by local toxicants) as well as ecological potentials in the local context. It is advisable to develop methods for practical risk assessment and risk management for cases of existing diffuse contamination, for example by developing a toolbox for site-specific risk assessment. Other methods can be envisaged. Specifically, rather than focusing mainly on compound characteristics, such methods should address ecology better than before. Partly based on the integration research, the Programme Committee of SSEO prepared a summary document (*Slotdocument*), elaborating on policy recommendations. This document was then used as basis for discussions with stakeholders (amongst others from various ministries and local authorities and from various non-governmental organizations), to derive realistic policy implications and define urgent research and policy needs.

Higher-tier methods to assess the implications of diffuse contamination can be scientifically underpinned (e.g., by the methods applied in the SSEO programme). To facilitate the use of such methods, a scientifically based toolbox for site-specific risk assessment[3] can be envisaged, in which models and measurement approaches are made available for broader, practical use (e.g., to make a site-specific evaluation of cases of diffuse contamination). Models and measurement techniques need to be formulated so their use limitations are known. Neither models nor measurement techniques should be applied beyond their limits of use. In SSEO, various models and approaches were the subject of a validation study, and this resulted in improvements of some models. All models and methods considered can be part of the toolbox, whereby it should be acknowledged that some models over-predict exposure and effects. As a whole, however, the set of validation studies is limited, but many models have a proven legacy of providing useful insights. Therefore, some have reached the status of "validation by adoption in practice".

From the viewpoint of national and international chemical regulation, the generic models for exposure and effects assessment have been formally adopted, for example in Guidance Documents. It is likely that novel scientific insights (like those generated by the SSEO programme) will lead in practice to a continuous evolution of the generic evaluation methods and regular revision of the quality criteria. From the viewpoint of authorities responsible for diffusely contaminated sites, a toolbox for site-specific risk assessment, or other practical methods, can be of great practical value provided that the generic risk assessment is always, or at least in most cases, a relatively conservative "signal" for using it. Such a toolbox should provide better insight into local exposure and the type and magnitude of local effects. The scientific output requires a regulatory framework for interpretation. Similar to establishing quality criteria, there is an aspect of regulatory value judgement, in this case on the acceptability or non-acceptability of local risks and effects, in comparison with the effects of other stressors and the context of the situation. The toolbox for risk assessment can help to merge classically separate scientific and regulatory fields, thereby supporting a clear regulatory need for more integrated assessments and the requirement that has evolved[4] to execute cost-benefit analyses.

There are two major regulatory issues that arise from the confrontation of the results of the research programme with current regulations. First, toxic compounds are addressed in various nationally and internationally adopted regulations, including preventive chemical-oriented policies, site and compartment-oriented policies and species and ecosystem-oriented policies. In recently-adopted regulations, the classical approach, where the environmental compartments must have a chemical quality that meets regulatory criteria, contains an aspect that has become much more important: Good Ecological Status. Second, given the increased importance of Good Ecological Status, there is an urgent need to establish appropriate linkages between the regulatory domains of chemical compounds, and site- and system-oriented approaches. Ecological quality is, and will always remain, the only universal currency which allows the avoidance of any "comparing-apples-to-oranges" problem: an increased ecological weight in a tiered system should be considered to solve site-problems with diffuse pollution. Ecology-based tiered systems must be embedded in the frameworks of all regulations at the same time. If not, national policy decision-makers and site managers will be confronted with conflicting results. The summary activities of the Programme Committee (*Slotdocument)* and of a practice-oriented working group have already focused on these crucial aspects.

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[3] The Dutch government has adopted such a toolbox in their new soil policies. See: RIVM, RIZA, Alterra. 2007. Risicotoolboxbodem. Bilthoven: RIVM.

[4] EZ/VROM/Justitie. 2003. Effectbeoordeling voorgenomen regelgeving. Ministeries van EZ, VROM en Justitie. Report nr 03ME19.

Frequently Asked Questions

This report considers a simple problem definition – what are the field effects of diffuse, low-level exposure to toxic compound mixtures in ecosystems – and a complex set of scientific answers, in the form of an initial review of a comprehensive set of original studies and findings from the SSEO programme. A section on Frequently Asked Questions (FAQs) has been added to facilitate quick cross-referencing through this report and especially to facilitate those who are interested in its possible policy implications.

These FAQs themselves are of a *scientific nature*, since this report resulted from reviewing the scientific findings of the programme. The FAQs follow a different line of reasoning than the report text. They are posed from the imaginary viewpoint of a scientist or a regulator who wants to acquire insight into some key issues that are at stake at the interface between science and regulation and in the scientific debate. This report does *not* provide *regulatory-oriented* FAQs and answers. For this, readers should refer to a list of such FAQs and answers as prepared by the SSEO Programme Committee and the SSEO Steering Committee (SSEO Programme Committee 2007). Both lists taken together should provide (1) the scientific questions and answers (that followed from the problem definition of the SSEO programme and the research done for this report) and (2) the regulatory answers based on those findings, which were derived by matching the findings with regulatory practices and innovations.

Regulatory problem definition and regulatory context

Q1: What is diffuse, low-level exposure to toxicant mixtures, and do situations of low-level mixture exposure regularly occur in the field?

A1: Diffuse low-level mixture exposure to toxicant mixtures is defined as the occurrence of field situations in which the regulatory criterion for a “clean environment” is exceeded. That is, the Target Values have been exceeded. Diffuse pollution can consist of one compound, but usually concerns mixtures. The exceedance of the “clean environment” criterion can be small or large, up to concentrations that trigger remediation (Intervention Value). National inventories of soil, sediment and water contamination show that vast areas of land and vast volumes of sediment and water can be characterized as sites where the “clean environment” criterion has been exceeded[5].

Q2: Do we have accurate regulatory methods to cope with the frequent occurrence of diffuse, low-level mixture exposure in the field?

A2: No. The focus has always been mainly on preventive and curative policies that are based on the ecotoxicity of the compounds, with insufficient options for accurate risk management of existing cases, and with an emphasis on suggesting risk management options for such sites.

Q3: Is the focus of the past – leaving risk management of low-level exposed ecosystems rather undefined – understandable?

A3: Yes. When the criteria for clean soil, sediment or water, and for remediation urgency were established, it was absolutely not expected that the application of those values would imply a need for risk management for a large number of low-level exposed ecosystems.

Q4: Has the regulatory problem definition changed?

[5] For example, inventories of sediment contamination (AKWA. 2001. Bagger in Beeld. Basisdocument Tienjarenscenario Waterbodems. 01.014, AKWA) and of candidate sites for soil sanitation (Kernteam Landsdekkend Beeld. 2004. Lands Dekkend Beeld. Eindrapport nulmeting werkvoorraad bodemsanering, and MNP (2007) MKBA-BoSa.

A4: Yes. Prevention and curation are maintained as regulatory principles, but are now seen in a broader context, which includes so-called MET and BET tests that are required for novel policies[6]; this involves : reviewing the environmental impacts of regulatory measures in addition to societal/budgetary reviews[7]; this tendency translates into a search for a more integrated view (environment, society, spatial planning, etc.), as well as for the balance between environmental benefits and cost effectiveness of risk management measures.

Q5: Could science, at the start of the SSEO programme, provide a scientific underpinning for regulatory approaches to handle the environmental impacts of low-level mixture exposure?

A5: No. Most regulatory methods were derived from the basic scientific principles that were used for setting environmental quality standards for separate compounds; this involves a generic risk assessment approach, with no attention for site-specific modification of risks. There was little room to account for variance in availability and the occurrence of mixtures, and little for considering specific sensitivities of exposed species or species groups.

Scientific problem definition and scientific context

Q1: Are the effects of low-level exposure of ecosystems to mixtures of toxicants predictable from exceedances of generic quality standards?

A1: No, or only to a limited extent. The generic standards *do* make a difference in the regulatory sense, namely the discrimination between clean and not-clean (with reference to the Target Value), or between contaminated and seriously contaminated sites (with reference to the Intervention Value). They *do not* serve as predictor of type and degree of impact.

Q2: Why do generic quality standards not serve as predictor of type and degree of impact?

A2: SSEO research and other research strongly suggests that the ecological responses of biota to toxicant mixtures, at any exposure level, are determined by the combination of (a) the local mixture (and the toxicity of its compounds), (b) the matrix (which determines the availability for uptake and exposure, through sorption), and (c) the exposed species or species group (which relates to intrinsic sensitivity). Further, field effects also depend on temporal and spatial aspects of exposure and species distribution. In other words, each situation is unique (though it can be successfully addressed, see Question/Answer 4).

Q3: Does SSEO or related research provide a strong underpinning for the latter answer?

A3: Yes. Field research executed at the three study sites, and extended with data from other, similar studies, has shown that responses range from small effects at high exposures (exceedances of intervention values) to large effects at low exposures that are considered rather safe. An example of effects at levels considered safe is provided by a study on the decline of butterflies in a Dutch nature reserve, where the decline is attributed to a sensitive cascade of ecological effects of mixture exposure. In that study, metal exposures influence host plant vigour, which indirectly influences butterfly populations.

Q4: Is there a standard, practical way address the problem, when each situation is unique?

A4: Yes. The standard risk assessment paradigm can be applied. The standard risk assessment paradadigm consists of (a) the problem formulation, (b) an assessment of exposure, (c) an

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[6] EZ/VROM/Justitie. 2003. Effectbeoordeling voorgenomen regelgeving. Ministeries van EZ, VROM en Justitie. Report nr 03ME19.

[7] BET=Bedrijfs-Effect Toetsing (check on the societal and economical impacts of new regulations; MET=Milieu Effect Toetsing (check on the environmental impacts of new regulations)

assessment of hazard (the exposure-effect relationship), and (d) the combination of b+c in a risk characterization.

Q5: What is then the difference between generic and site-specific risk assessment when the application of the risk-assessment paradigm is the same?

A5: The differences are:
(a) the regulatory problem definition differs (e.g., “what is a safe level for compound A” *versus* “what is the local impact of mixture X in soil Y on species group Z”),
(b) site-specific sorption to the matrix is taken into account the exposure assessment only in the latter case, and
(c) the exposed species or species group is taken into account only in the hazard assessment only in the latter case. As a consequence, the risk characterization changes from generic to specific.

Q6: Which problem formulations have been posed to the risk assessors during the SSEO research period?

A6: There are many. The generic problem formulation still exists; for example it is still the basis for evaluating novel compounds. Generic exposure and effect-assessment scenarios are combined to generate a generic risk characterization of the compound under investigation. From this result, it can be decided whether there is a regulatory problem. Specific problem formulations for sites with an excessive contamination level can be a request to develop standard assessment protocols (to address the risk of, for example, sediment deposition on land[8], the re-use of secondary building material[9], or soil management[10]) or a request to evaluate whether populations of protected target species are endangered by local low-level mixture exposure. In this case, site-specific information can be used in the exposure and effects assessment, resulting in a more specific insight from the risk characterization.

Q7: What methods are available for site-specific risk assessment?

A7: SSEO research and associated research show that both models and measurements can be applied in exposure and hazard assessment, and thus can be used for risk characterization.

Q8: Is there currently sufficient guidance on the use of models and/or measurements, given the increased variability of problem formulations?

A8: No. However, there are national and international projects focusing on the development of a risk assessment toolbox, and recently a generalized guidance proposal has been prepared during an international workshop (EXPECT[11]).

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[8] Posthuma L, De Zwart D, Wintersen A, Lijzen J, Swartjes F, Cuypers C, Van Noort P, Harmsen J, Groenenberg BJ. 2006a. Beslissen over bagger op bodem. Deel 1. Systeembenadering, model en praktijkvoorbeelden. Bilthoven, the Netherlands: National Institute for Public Health and the Environment. Report nr 711701044.

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[10] VROM. 2006b. Concept-Besluit Bodemkwaliteit. Concept 17 maart 2006. Den Haag, The Netherlands: Ministerie VROM.

[11] Solomon et al. (in press) Extrapolation Practices in the Effect Characterization of Toxic chemicals. SETAC-Press; Posthuma L, De Zwart D, Solomon KR, Brock T. In Press. Guidance on the application of extrapolation methods in ecological exposure and effects characterization of chemicals. In: Solomon KR, Brock T, De Zwart D, Dyer SD, Posthuma L, Richards S, Sanderson H, Sibley P, Van den Brink PJ, editors. EXPECT: Extrapolation Practice for Ecotoxicological Effect Characterization of Chemicals. Boca Raton, FL, USA: SETAC Press. Chapter 10.

Q9: Are there guiding principles for toolbox design?
A9: Yes. The toolbox should contain models and measurement approaches that allow risk assessors to address all current problem definitions in an efficient and reproducible way; the models and measurements should (preferably) be validated, and method strengths and limitations should be known. Next, the toolbox should allow for cost-effective use of the models and measurements, i.e. it should be constructed according to a tiering principle.

Q10: Why should there be a tiering system in risk assessment?
A10: For cost-effectiveness in practical use. Simple, repetitive problem formulations, and problem formulations posed in a preventive context, can in practice only be built on simple, cheap, and "non-data hungry models, that therefore yield conservative answers, and that therefore can be applied to many cases. Refined problem formulations, e.g. on the protection of target species or on local mixture risks, require refined, more data-hungry methods that provide more precise insights into local risks, and that are therefore more useful for site management.

Specific scientific findings of SSEO and similar research lines

Q1: Which aspects of exposure assessment were demonstrated in the SSEO research to be relevant for site-specific risk assessment?
A1: There are two major aspects: local natural background concentrations and availability of compounds for uptake as determined by the matrix and/or by food-chain transfer. Knowing the local natural background of a contaminant may be of help in defining the need for remediation. In some cases, local contamination may be fully attributed to natural causes, and this may imply a different risk management strategy as compared to recent, man-made contamination. A key example of modification of availability by matrix components is black carbon, which strongly influences exposure to organic contaminants. Accumulation measurements need be performed in case insights are needed into true exposure levels; firstly because exposure and actual uptake can be easily proven for the local biota for many compounds, and secondly because this can help to identify which compound(s) contributed to adverse effects, and thirdly because the occurrence of biomagnification can be estimated and considered.

Q2: Are there more aspects concerning exposure?
A2: Yes. Although not specifically addressed in SSEO, the regulatory problem formulation can be translated to a research problem formulation on the basis of a local system analysis: are there other sources of increased exposure (e.g., extra exposure resulting from contaminants present in rainfall or in manure added to soil) or reduced exposure (e.g., breakdown of organic compounds).

Q3: Which aspects of hazard assessment were demonstrated in the SSEO research to be relevant for site-specific risk assessment?
A3: The hazard assessment step in a risk assessment considers, in fact, the concentration-response relationships of all relevant compounds and exposed species. The SSEO research has demonstrated a large variability across species in their sensitivity to exposure.

Q4: Are there remaining uncertainties on hazard and risk?
A4: Yes. Species sensitivities may not be fully known; laboratory measurements on test endpoints need not represent the population viability endpoint that should have been measured (e.g., the No Observed Effect Concentration (NOEC) for reproduction need not represent the NOEC for population decline). Furthermore, population viability may be affected via ecological

interactions (e.g., the butterfly study cited above and an example of a protected bird, the godwit). Next, when protection not only concerns species, but also food webs, it appears that food web sensitivity may differ from the expectations from single-species assessments. Finally, other stressors may increase or decrease the sensitivity of exposed organisms.

Q5: Are exposures easy to quantify in field conditions?
A5: Yes. Compared to proofing effects of mixtures, the evidence for exposure in field conditions is relatively easy, due to similar "currencies" (mg/kg of a compound, and similar units to express a concentration).

Q6: Are effects easy to quantify in field conditions?
A6: No. Effects can be measured in an array of "currencies" (abundance change, biomass change, the occurrence of community tolerance (PICT[12]), functional changes, et cetera). Most of these are naturally variable over time and space, and are influenced by many factors.

Q7: Which scientific solutions are proposed for identifying effects in field conditions?
A7: There are various solutions. In purely empirical (observational) studies, appropriate sampling designs – like a gradient approach – need be followed, whereby care is taken to address both "true" exposure (by measuring body concentrations) as well as major confounding factors that co-influence biotic responses. However, environmental samples can be subject to experimental treatment – as in the community-tolerance studies (PICT) – which strengthens inferences on the occurrence and cause of effects. However, it remains extremely difficult to provide full proof that mixtures are the cause of ecological impacts in field conditions. Finally, a weight-of-evidence (WOE) approach can be proposed, i.e. applying various approaches to gain insight into site-specific exposure and effects.

Q8: What is a Weight-of-Evidence (WOE) approach?
A8: A WOE approach starts from the principle that (related to the difficulties described above) multiple lines of independent evidence should be followed to obtain an integral view of the likeliness of local effects. The Dutch soil quality triad is based on WOE, and follows a standardized decision scheme, identifying which methods should be applied according to a tiered system.

Q9: Does failure to prove effects imply absence of effects?
A9: No. Firstly, the local exposure may be too low to imply effects. However, if that is not the case, such failures can relate to limitations of the study design and sensitivity of the endpoint. Two-location comparisons, gradient studies without consideration of confounding factors, or low-replicate studies may fail to demonstrate effects due to an inappropriate design and/or low statistical power. Secondly, it should be acknowledged that the sensitivity of response endpoints generally decreases from the level of cellular (biomarker) reponses (as early warning systems), via the population-level (abundance) changes to the community-level (change of biodiversity or function). Thirdly, toxic effects may be masked by major other stressors (like flooding effects on earthworm populations).

Q10: Is the risk assessment paradigm now sufficiently defined?
A10: No. SSEO research and associated research has clearly identified that, besides exposure and hazard assessment, two more aspects need be clearly identified and incorporated in site-

[12] Pollution-Induced Community Tolerance

specific assessments: spatial and temporal aspects. Both aspects may modify exposure as well as effects and recovery, and need be incorporated in site-specific risk assessment when the regulatory question focuses on areas rather than on compounds.

Specific scientific findings of SSEO model validation studies

Q1: Do the models studied in the context of SSEO provide "the full array of models" available?

A1: No. There are more models and approaches that can be used to address site-specific exposure and effects assessment as well as site-specific risk assessment. A key item is that models and approaches should be used with explicit motivation: "Why use this model or approach for this type of problem formulation?". This implies knowledge of the strengths and weaknesses of a model. The research works of the SSEO programme have resulted in better insights into the applicability of models and into possible models being systematically biased to conservative outcomes. It should be noted that systematic conservatism need not be a bad characteristic for problems where the alternative approach would be a safety factor.

Q2: Did the SSEO model validation studies give *final* answers on model validity and robustness?

A2: No. Model validity and robustness are now better known for the models studied. To reach the goal of *final* answers on the validity and robustness of some models, the data of the SSEO programme should have been collected more systematically, so that it could have been established that a model makes accurate predictions in an array of different situations. The available data set was too small for that, due to the focus on three study areas. On the other hand, this focus implies that there were better data for this purpose than would have been collected in a programme design with free choice of sites and study objects.

Q3: Did the SSEO model validation studies give relevant information on model use?

A3: Yes. Various strengths and weaknesses were identified for each model, and various models were improved on the basis of SSEO study data.

Possible implications for regulations

Q1: Has it been established that ecosystems in the Netherlands are exposed to a diffuse contamination by mixtures of different compounds?

A1: Yes. It has been shown that the studied three areas (a floodplain, a tidal area and a peat area with "toemaakdek", which is a layer of medieval household waste) not only contain mixtures of chemical compounds to levels exceeding the definition of "clean systems", but also this leads to true exposure of ecosystems in those areas to those compounds in various degrees. Exposure and effects are variable, due to unique combinations of local mixtures (composition and concentration level), the local matrix characteristics (soil, water or sediment type) determining sorption, and sensitivity of exposed species and species groups. This finding is relevant for the following regulatory contexts:

- For evaluating national protection criteria as formulated in the context of chemical-oriented policies
- For evaluation of site-specific risk management options in case of existing diffuse contamination for which cleanup is not a realistic management option
- For evaluation of options for species and habitat protection in case species inhabit or visit areas with diffuse contamination
- For international obligations, formulated in various treaties (from global, like UNCED, to European, like the EU-Water Framework Directive)

Q2: Is exceedance of chemical-related generic quality criteria a clear indicator for the presence and magnitude of ecological effects?

A2: No. This relates to the way those criteria have been established. First, legal criteria are often the most stringent result of two assessment, namely an ecological and a human-oriented risk assessment. This means that the exceedance of a criterion might imply human risks only. Furthermore, the abovementioned unique combinations of mixture, matrix and exposed species or species groups are important. But the major reason is that the criteria were not designed to be used in this specific way. Exceedance of quality criteria is an indicative signal that regulatory-defined unacceptable risks may occur. However, the translation of the exceedance of *risk* limits into probable ecosystem *effects* (defined in kind and magnitude) requires additional information from the specific site.

Q3: When ecosystem effects in the field become very specific as a consequence of mixture-site-species combinations, can proposals be made to improve chemical regulation, i.e., decrease the impacts of chemicals released or present in the environment?

A3: Yes. A novel approach is to look at options for site-oriented risk management, in addition to chemical-oriented preventive and curative policies. The original, chemical-oriented approaches have yielded methods for prevention and curation. These approaches can be maintained to avoid further diffuse contamination. In addition, for the intermediate exposure levels (for sites neither clean nor requiring urgent sanitation), site specific or area specific management strategies could be developed to make management decisions that are both environmentally sound and cost-effective. The derivation of such strategies will be possible when appropriate site-specific methods for risk quantification are implemented. Their application will be possible when there is a tripartite regulatory framework: prevention – management – curation.

Q4: Did the SSEO-programme result in indications to suggest generally higher or lower quality criteria?

A4: No. The quality criteria can, in general, be considered sufficiently protective to the level defined as the key protection endpoints of chemical policy, i.e. structural and functional integrity of ecosystems. There are some exceptions to this, for example acute side-effects of pesticide use on non-target organisms, or the possible decline in butterfly populations as a consequence of a sensitive ecological cascade (from metals, via host plant vigour, to decline of butterfly species) in a nature reserve area in the Netherlands. Changing the generic risk assessment scenarios that are used to derive the criteria is, however, can be one of the options to think of. Scientific insights increase, and it is imaginable and realistic to expect that the exposure and effects scenarios of the generic frameworks will change. Implementing major changes (like generally higher or lower criteria) will probably be difficult due to the high degree of standardization and acceptance of current methods, i.e. there will likely be process-oriented problems. Moreover, novel methods will yield novel criteria, which in turn would again require validation to the level that the protection targets have reached.

Q5: Is there a reason to believe that there are major synergistic effects between diffuse pollution with chemical mixtures and other stressors?

A5: A dual answer is needed here. For the quality criteria, it has been concluded that they appear to offer mostly sufficient protection for ecosystem structural and functional integrity. This implies that synergistic effects between compounds in a mixture, and between mixtures and other stressors, are apparently “handled” in the general exposure and effects scenarios of the generic assessments. For site-specific assessments, the data from SSEO have shown that the net effects encountered in field ecosystems always depend on mixtures (composition/concentration – matrix – species) in concert with other stressors. This was shown in eco-epidemiological research. Apart from evidence that more factors together influence biotic integrity, SSEO did not present clear evidence on the presence or absence of synergistic effects between mixtures and other stressors, i.e. it is unclear whether the net responses are larger than expected from non-synergistic influences only.

Q6: Can and must linkages between chemical-oriented and site-, species-, and habitat-oriented policies be established, and can an apparent feeling of "misfit" between both types of regulation be solved?

A6: Yes. Linkage can be realized through the application of a tiered system for risk assessment. In the first, simple and (most often) conservative tier, the generic risks of singular compounds for the ecosystem in water, sediment or soil are to be established, and these efforts will result in either prevention or in the site-specific signal that an existing contamination is suspect. All this follows current approaches. For suspect sites, more site information can be entered into the exposure and effects assessment steps of a risk assessment, yielding more precise information on local risk and probable effect levels. This information is thereafter used to formulate management decision for the specific area, or (in the case of a workload of many areas that are to be managed) in prioritization of management actions between areas. The apparent feeling of "misfit" between chemical-oriented and site-oriented policies can be solved. This can be done by recognizing that prevention and curation methods remain important, but that *in addition and as secondary approach*, site-specific methods can be applied. By applying appropriate design criteria for the whole set of assessment methods, a tiered and internally consistent set of methods can be designed.

Q7: Are there international tendencies to support an additional, site-specific view of the problem of chemical pollution?

A7: Yes. In particular, the EU Water Framework Directive applies two Lines of Evidence for judging water quality. The first is to look at exceedance of quality criteria for a selected set of compounds. In addition, however, the link to the protection endpoint is made very explicit; water bodies are eventually evaluated according to the concept of Good Ecological Status (GES). This is done because Good Ecological Status depends not only on meeting the quality criteria for the selected compounds, but also on many other factors, like physical and chemical habitat characteristics, geography and biological characteristics of species. GES is a typical site-specific or catchment-specific concept, and it involves the need to use ecology-based approaches.

Q8: Are there methods to do practical site-specific risk assessments in case site managers have indications of site pollution?

A8: Yes. There is an array of methods including modelling techniques, measurement techniques or combinations of both. An example of the latter is the triad approach, whereby three Lines of Evidence (chemical, bioassay and field assessments) are combined in a Weight of Evidence approach. As an option (which has been realized for soil management as part of new soil policies in the Netherlands), operational methods can be compiled in a toolbox for risk assessment. Additional options can be envisaged, and these could mainly consider which approaches can be taken for effective site management. Regarding the source and pathway aspects, this will put the focus on environmental chemistry: which methods are available to reduce exposure, and thereby impacts, if sanitation is not realistic? And what is the likely consequence of changes in soil use according to chemical behaviour scenarios? For the receptor aspects, this will put the focus on ecology and ecology-based scenarios for recovery.

Q9: What is the key message that can be derived from the results of the SSEO research to improve regulations?

A9: When it is assumed that

- chemical regulations have recently been renewed (EU-REACH), and
- there is a major need for site-specific assessments to support practical management of contaminated sites,

the key message is that there are sound scientific and practical principles to design and generalize approaches for site-specific assessments (the tier approach), whereby appropriate methods to quantify stressors (both mixtures and other stressors) and sound ecological approaches can be combined to optimize management of sites with existing diffuse contamination. The innovative aspect is that one doesn't look at things that are *limited* by the degree of absolute pollution (through the quality criteria), but at things that are *possible* at diffusely contaminated sites, despite the absolute degree of pollution.

1 General introduction

By: L. Posthuma, M. G. Vijver & F. Kuenen

1.1 Risks, modelling and environmental policies

Chemical compounds are emitted into the environment due to human activities. These compounds may cause adverse effects on humans and ecosystems. This triggered national governments to develop various policy approaches. General policies focused on preventing the occurrence of adverse effects of the compounds, often with the characteristics of the toxic compounds themselves as a starting position. This resulted in chemical regulation, and very recently (June 2007) in the adoption of EU-REACH, which regulates novel chemical compounds in the European Union. These approaches can be considered successful, since major chemically induced environmental impacts have declined in both frequency and intensity since the 1960s and 1970s. At the same time, chemical compounds are part of other regulations, like those focusing on specific environmental compartments (water, soil) and on the protection of biological species, their habitats and ecosystems. The target of those policies is to avoid reductions of environmental quality and to limit and reduce risks and effects of toxic compounds and their mixtures, as well as those of other stressors. And from this perspective, many sites can be considered contaminated. Recent inventories showed that large parts of the Netherlands cannot be considered clean (AKWA 2001; Kernteam Landsdekkend Beeld 2004). These inventories implied that the regulation of chemicals is not yet sufficiently controlled.

By the late 1990s, it became apparent that vast volumes and areas of the country were exposed at contaminant levels higher than the so-called Target Values (identifying a clean environment). Preliminary findings triggered the design of a major research programme, the Dutch Stimulation Programme System-Oriented Ecotoxicological Research, abbreviated in Dutch as “SSEO” (NWO 1999).

The targets of the SSEO programme were:

- to gain scientific insight into the risk of chronic exposure of ecosystems to a combination of pollutants, with or without the influences of other stress factors,
- in order to eventually improve environmental management of toxicants

The programme ran between 1998 and 2006, and was completed in 2007. The present report considers the major scientific results of this programme. It was prepared on the basis of the research done by a group of scientists on the issues of (1) the validation of ecotoxicological models, and (2) the integration of SSEO findings (for project description, see Posthuma et al. (2001)). The scientific findings, published in more than 100 original research publications, were thus compiled and integrated in the present report. Moreover, this scientific integration has been recently extended with practice-oriented publications commissioned by the SSEO Programme and Steering committees. This has resulted in two documents that describe the implications and optional usages of the programme results for a range of current policies (Eijsackers et al. 2007; SSEO Programme Committee 2007). In this way, a continuous link between original scientific findings and operational use has been established.

The present report integrates the findings from the programme and links the findings to current policies. This implies a focus on risk assessment. In the Netherlands, Europe and elsewhere, the chemical-oriented policies are founded on the choice to use a risk-based approach in these regulations (see VROM, 1988 and Van de Meent et al., 1990). The use of a risk-based approach in environmental policies has been advocated for following reasons, which have been adapted from Suter (1993):

1. formal risk assessments require an explicit identification of protection and policy targets; these are the starting point for any risk assessment
2. they require the clear definition of approaches and assumptions of the risk assessment process, to establish a clear background for discussions about the management of different risks
3. they require a clear division of roles between the scientific process of risk assessment and the policy evaluation of risk management
4. they are a systematic basis for improving recognition and understanding of the occurrence of risks and effects
5. they allow for the comparison of risks induced by different stressors and for priority setting in risk management
6. they show the explicit uncertainties that are embedded in the forecasting of events.

Risk is usually considered the product of the probability of an adverse effect and the magnitude of such an effect. Risk assessment, therefore, usually consists of modelling. Scientific models of exposure and effects, therefore, are fundamental to an array of policies, and modelling specifically plays an important role in the regulation of toxic compounds.

Modelling can, however, yield good or bad results. Validation of models is thus very relevant for decision making. Models that tend to over-predict the impacts of toxic compounds will lead to low cost-effectiveness of measures that are taken on the basis of model results that predict adverse effects where there aren't any. Models that under-predict effects will lead to adverse effects where they are neither expected nor considered acceptable.

1.2 Aims and approaches

This report addresses the validation of ecotoxicological models that are in use for the regulation of toxic compounds, both in the compound-oriented policies as well as the other policies. It also focuses on a limited integration of the findings of the SSEO research programme, partly by using the compiled data and by placing the findings of the programme in a broader (policy) context. The over-all target of the research work underlying this report was to integrate the findings of the programme in order to formulate options for improved, integrated policy approaches in preventing and handling risks of toxic compound mixtures under realistic field conditions.
The approach that was followed consisted of:

1. compiling the data of the researchers in the SSEO programme
2. validating models using the compiled results to investigate the degree of validity of selected models, and
3. initially integrating the programme results and disseminating the results by obtaining an overview of SSEO results and formulating options for applying the results of the programme in the regulatory context.

For this report, original research data and research papers, either published, or in press, were used. The research papers should be consulted for additional details; extensive literature details are provided. The data mainly originate from the three study sites selected for the SSEO programme. Where appropriate, the findings obtained from within the context of the programme are extended with published studies funded by other sources, so as to avoid erroneous (oversimplified or over-generalized) interpretations.

1.3 Reader's guide

This report consists of 5 Sections and 33 Chapters.

- Section 1 introduces the SSEO programme and the characteristics of the SSEO study sites. It also presents a broader introduction to some key aspects of risk assessment and model validation.
- Section 2 describes the studies on the validation of ecotoxicological models with SSEO data.
- Section 3 elaborates on these studies by looking at major findings from SSEO projects that didn't look at model validation.
- Section 4 elaborates on both issues by looking at two examples in which the role of diffuse pollution has been analysed from available, large monitoring data sets
- Section 5 presents a general discussion of all relevant issues, presents options for regulatory use of SSEO results and formulates the key conclusions.

Section 1. The background of the SSEO and this study

The chapters in Section 1 describe the broader context of this report. The present report was commissioned as part of the SSEO programme, a stimulation programme focusing on the exposure and field effects of diffuse pollution by mixtures of contaminants at relatively low concentrations. To make the results of the programme useful for regulatory application, one project (Posthuma et al. 2001) was funded to look at the validity of the models used in regulatory risk assessment and at the integration of the scientific results of the programme as a whole. The key aim was to analyse these issues so that the scientific results of the programme can be practically used in the regulation of toxic compounds and pesticides, and in the risk management of contaminants in water, soil, sediment and in nature and species protection.

2 System-oriented Ecotoxicological Research: background information

By: L. Posthuma and H. J. P. Eijsackers

In the mid-1990s, after a period of use of generic soil, sediment and water quality criteria, it appeared that many questions remained of the “what-if” type: “What happens in exposed ecosystems in the field in case of diffuse pollution?” It became apparent that at many sites ecosystems were exposed to toxicant mixtures that exceeded the values that were in use to characterize clean soil, sediment or water. But those sites were not polluted highly enough to trigger remediation.

In 1996, the Dutch Government asked the Advisory Council for Research on Spatial planning, Nature and the Environment (Raad voor Milieu en Natuur Onderzoek, RMNO) what kind of research was necessary on this subject. The Council issued a report (RMNO 1996) that described the regulatory motives for and outlines of a stimulation programme to unveil the possible problems of the so-called “grey veil” of contaminants: diffusely spread cocktails of chemical compounds that occur in Dutch ecosystems.

In 1999, the Netherlands Organisation for Scientific Research (NWO) issued a kick-off brochure for this programme (NWO, 1999). The brochure described a two-phase approach for the programme – similar to that proposed in the RMNO document. An initial phase of research should be followed by more in-depth studies in a second phase. NWO should organize the SSEO programme in a scientifically sound way, i.e. via the selection of the most appropriate research proposals as submitted for both phases. As a result, an array of projects was funded, in part organized in sub-programmes that consist of various projects, for the period of 2000-2006. The work was to be executed by PhD students and post-docs, and was to be supervised by the senior scientists who wrote the proposals.

The kick-off brochure also mentioned the need for an over-arching project, which should focus on the integration and the translation of the scientific findings with regulatory practice. The current report is the main result of the project that was proposed for the integration step (Posthuma et al., 2001), and which was funded by NWO. The current report focuses on the validation of models that are used in the context of the various regulations, namely: toxic compound regulations, pesticide regulations, compartment-oriented regulations (soil, sediment, water) and nature and species protection regulations. It provides further a first integration of the results of the research programme. In a previous report (Posthuma et al., 2005), we described some of the models that could be selected for the validation studies.
The kick-off brochure of NWO, as well as the RMNO report, also mentioned that the choice of compounds and study sites was key to the issue of integration; if all studies were independent regarding choice of compounds and study sites, the added value of the programme as a whole would be limited – especially considering the enormous array of possible studies that could be proposed. The expected variance is evidently related to the number of possible compounds (>100,000) and substrate and ecosystem types that could be the subject of study. To obtain maximum added value, the programme focused on compounds which were considered a major regulatory problem and for which sufficient scientific data are already available (Nentenaar and Rip, 1999). Moreover, the programme should be executed in three study areas that fulfilled two major criteria: first, the criterion of contaminant gradients being present, and second, the criterion that the sites should have regulatory relevance as example sites – representing a relevant set of problem sites. For the full set of criteria, see Table 1.
In the subsequent chapters, we introduce the study sites, and the major findings for those sites. The remainder of this report concerns the validation and integration targets.

Table 1 Criteria for the selection of research locations for the SSEO programme.

Issue #	Criterion
1	Locations must be characterized by the presence of a gradient of contamination (including nutrients), with concentrations to the intervention value. The contamination must have diffuse characteristics.
2	The sites must be characterized by variability in soil or sediment characteristics (such as pH, humidity, clay content, organic matter content), so that the role of this variability for determining "bioavailability" can be investigated.
3	Variation in ecosystem types: there must be both an aquatic and a terrestrial system in the subset of study areas
4	The study areas must be of regulatory relevance, either due to current characteristics or due to the vicinity to parts of the Ecological Main Structure of the Netherlands
5	For various aspects of the sites (type of contamination, exposed species and ecosystem types, modifying factors, food chains, keystone species, keystone interactions, functional redundancy) there should preferably be some basic information. This is key, in view of the integration of eventual findings of the programme through model analyses.
6	Each study area must have reference sites to which results can be compared. The reference condition can be defined by either a clean location with similar characteristics (apart form the contamination) or the "end" of a gradient, as an internal reference for an area.

3 Site characteristics and research at site "the Biesbosch"

By: C.A.M. van Gestel, T. Hamers, P.H.F. Hobbelen, J.E. Koolhaas & M.J.M. Notten

3.1 Site characterization and contamination

The Biesbosch is the delta of the Rhine and Meuse rivers (Figure 1). For the Netherlands, it is a unique freshwater tidal area. Soils of the Biesbosch floodplains typically have a high pH (pH-$CaCl_2$ 7.3-7.7) and high contents of clay (15-45 %) and organic matter (15-30 %).

In the period 1955-1980, the rivers were highly polluted, and so were the sediments that were deposited in the Biesbosch. As a consequence, the floodplain soils contain high concentrations of metals, e.g. cadmium (10-25 mg/kg DW), copper (60-300 mg/kg DW), and zinc (650-1700 mg/kg DW) (Hobbelen et al. 2006b). Zinc levels in soil exceed the Dutch Intervention Values at all sites studied, and this is also the case for cadmium at most of the sites sampled. Concentrations of other metals, such as copper and lead, exceed the concentration levels that identify the condition of "clean sediment", but do not exceed Intervention Values. Biesbosch soils are also contaminated with organic micropollutants, such as Polycyclic Aromatic Hydrocarbons (PAH), mineral oil and chlorinated compounds (like PCBs and chlorinated pesticides). Concentrations of these organic compounds are generally below their Intervention Values, with the highest concentrations amounting to ∑PCB 0.3-1.2 mg/kg, ∑PAK 10-14 mg/kg and mineral oil 260-1100 mg/kg.

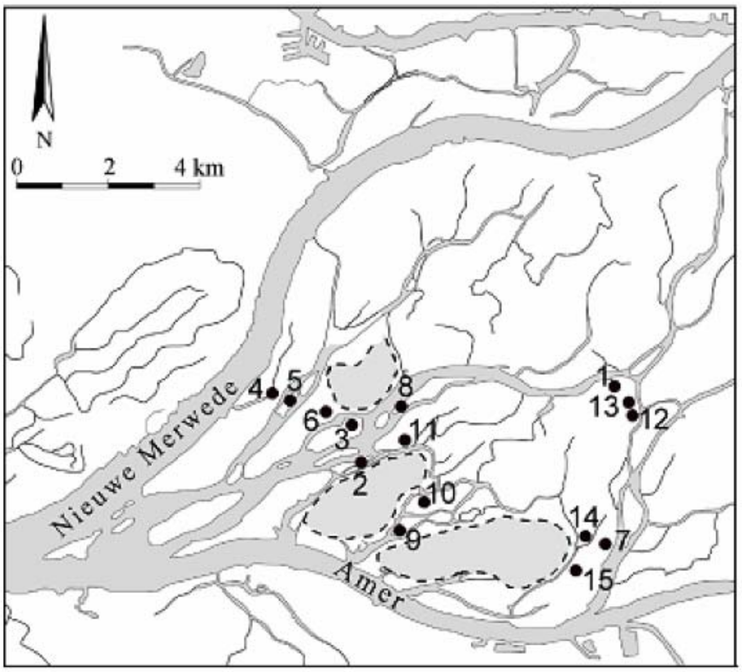

Figure 1. The SSEO research area in the Biesbosch. Areas within the dotted lines are basins used for the storage and pre-treatment of river water to be used for the production of drinking water. Numbers correspond to sampling sites as used in the original publications.

Two main questions were addressed for the study area of the Biesbosch:

1. How bioavailable are metals and organic pollutants in the Biesbosch soils and do they accumulate in the terrestrial food chain?
2. Are organisms living in the Biesbosch soils affected by pollution and does this affect functioning of the ecosystem?

3.2 Research spearheads and approaches

To determine bioavailability of metals and organic pollutants in the Biesbosch soils, bioaccumulation levels were determined in earthworms, isopods, millipedes, snails, plants and small mammals. In the case of earthworms, studies included 15 sites, representing a gradient of metal pollution (Figure 1; see also Hobbelen et al. (2006a)). For the other organisms, the focus mainly was on two sites, Lage Hof and Petrusplaat Oost; the first is one of the most polluted Biesbosch sites (Hobbelen et al. 2004; Notten et al. 2005). Exposure of small mammals was assessed using biomarkers of exposure, such as measurements of hepatic EROD and PROD activity and bulky DNA adducts. In addition, the DR-Calux® bioassay was applied to assess PAH levels in earthworms and snails, which are the major food items of carnivorous mice (See Hamers et al. 2006 for further details).

The possible ecological risk of pollution in the Biesbosch soils was estimated from the total and available chemical concentrations measured in soil. Total concentrations were compared with trigger values, such as the Dutch Intervention Values (see above). Available concentrations were compared with levels reported in the literature for polluted and non-polluted soils. Next, concentrations measured in the various organisms, reflecting bioavailability, were compared with background concentrations reported for organisms from non-polluted areas. Exceedance of background values was an indication of possible risk.

Effects on earthworms were determined by applying a biomarker, the Neutral Red Retention Time (NRRT) assay (Weeks and Svendsen 1996). This is a marker of general stress, which seems most responsive to metals, in particular copper (Scott-Fordsmand and Weeks 2000). Determining the composition of the arthropod community at two sites assessed the effects on the structure of the invertebrate community. Effects on the function of the ecosystem were determined by deploying litterbags and bait-lamina sticks in the field and relating decomposition and feeding activity to the biomass of detritivorous earthworms and millipedes. Bioassays were performed in the laboratory with the earthworm species *Lumbricus rubellus*, and included measurements of litter decomposition or bait-lamina consumption as well as the NRRT biomarker.

3.3 Main results

Metal concentrations in pore water or 0.01 M $CaCl_2$ extracts generally increased with increasing total soil concentration, especially for cadmium, copper and lead. Although total metal concentrations were high, available concentrations were rather low, and fell in the range of levels found in soils from non-polluted areas (Hobbelen et al. 2004; Hobbelen et al. 2006a).

Metal concentrations in earthworms from the gradient increased with increasing soil concentrations, but not with pore water concentrations. The soil-dwelling species *Aporrectodea caliginosa* contained higher metal concentrations than the litter-feeding *Lumbricus rubellus*. Despite the low availability in soil, earthworm cadmium and copper concentrations at the most polluted sites did exceed values reported for animals from non-polluted sites (Hobbelen et al. 2006a). Soil arthropods did not accumulate metals to a great extent, and in fact only copper levels in millipedes from the most polluted site exceeded background levels (Hobbelen et al. 2004). The most dominant plant species, the stinging nettle *Urtica dioica*, contained only very low metal concentrations, far below the maximum values found in plants from non-polluted sites. Nevertheless, the main herbivore feeding on these plants, the snail *Cepaea nemoralis*, did contain metal concentrations that were much higher than background values (Notten et al. 2005). In snails, cadmium was the most accumulative metal. For a further discussion on results on the accumulation of metals in earthworms, see the Chapter by Veltman et al.(Chapter 11).

The carnivorous shrew (*Sorex araneus*) showed increased metal levels in the kidney at both Biesbosch sites, while EROD and PROD activity were especially elevated at the most polluted site. Since this was not the case for the herbivorous bank voles (*Clethrionomys glareolus*), we concluded that the carnivorous feeding behaviour leads to an increased accumulation of metals in small mammals. Bulky DNA-adduct levels suggested exposure to a complex mixture of PAHs, but no differences between species and sites were found. The DR-Calux® bioassay showed higher Benzo(a)pyrene equivalency factor (BEQ) levels in snails but not earthworms from the most polluted site, suggesting an increased exposure level of PCBs at this site (Hamers et al. 2006).

Bioassays with *Lumbricus rubellus* showed that this earthworm species may reach similar levels of metals in the body after 4 weeks of exposure as earthworms collected from the same soils in the field (Koolhaas et al., in prep.). Earthworms (*Lumbricus rubellus*) collected from the most polluted site, Lage Hof, showed a reduced NRRT compared to animals from less polluted sites. This was also found for earthworms exposed for 4 weeks to soil from the same site. In both cases, reduction of NRRT correlated with an increase of internal copper levels in the earthworms (Koolhaas et al., in prep.). Earthworms kept in Biesbosch soils in the bioassays did show increased rather than decreased litter consumption rates with increasing metal concentrations in the soil (Hobbelen et al. 2006b), and no effects were seen on bait-lamina consumption rates (Koolhaas et al., in prep).

Litter decomposition was significantly and positively correlated with detritivore biomass and 0.01 M $CaCl_2$ extractable cadmium concentration in soil. Metal pollution at the 15 Biesbosch sites appeared not to be a major factor in determining detritivore species richness and densities, although biomass of the earthworm *Lumbricus rubellus* was positively correlated with available zinc concentrations in the soils (Hobbelen et al. 2006c). For a description of effects on litter decomposition, see Chapter 23.

3.4 Summary of findings for the Biesbosch research area

Pollution levels in the Biesbosch floodplain soils are high, but the availability of the pollutants in Biesbosch floodplain soils and sediments seems rather low. Nevertheless, metal concentrations, especially cadmium and in some cases copper, did exceed background levels in millipedes, earthworms, snails and carnivorous mammals. In addition, the mammals seemed to be at risk from exposure to PCBs. Effects on a biomarker in earthworms did not lead to effects on the population density or the functioning of earthworms in the process of litter decomposition. And no clear effects were seen on the community of detritivores (millipedes, earthworms) in a range of Biesbosch soils representing a gradient of metal pollution. Bioassays appeared to be useful tools of performing exposure studies with earthworms under controlled conditions.

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4 Site characteristics and research at site "Toemaakdek" in "De Ronde Venen"

By: M. Rutgers & J. Bogte

4.1 Site characterization and contamination

The landscape in the central-western part of the Netherlands has been shaped by peat digging activities and land reclamation in previous centuries. Nowadays, the region is characterized by mosaic patterns of eutrophic lakes, grasslands with remnants of old peat, and reclaimed polders with a floor of old marine and river clay (Figure 2). The region contains one of Europe's most important low peat marshlands, mainly with dairy farms and nature areas.

The area is a good example of a region that is contaminated with a grey veil of contaminants. In this area, typical soil pollution is encountered in the top layer of the soil. Until the beginning of the 20th century the grasslands in the peat area were improved via a land management method called "toemaken" (literally: to prepare). The method principally consisted of preparing a ripened mixture of farmyard manure and stable manure, dredged sludge, household waste from surrounding cities (e.g. Amsterdam, Utrecht, Leiden), and sometimes dune sand, and applying this mixture during a 4 year cycle to the grassland (Lexmond et al. 1987).

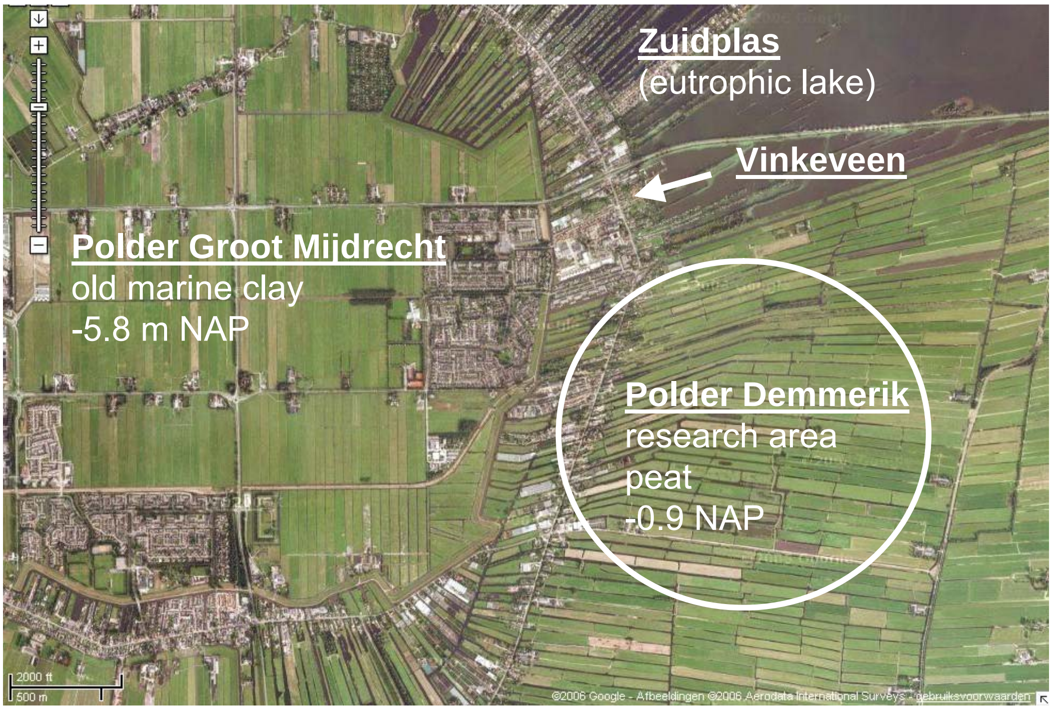

Figure 2. Satellite photograph of a part of the Ronde Venen area, with a characteristic mosaic pattern of eutrophic lakes (Vinkeveensche plassen), reclaimed marshland (polder Groot Mijdrecht) and grasslands on old peat (Demmerikse polder; east of Demmerik and south of Vinkeveen). Note different elevation levels of the polders, indicative for the soil type in this area (shown in m with respect to NAP).

This agricultural practice has led to a specific, well-moulded black A_1 soil horizon 15 to 50 cm thick, with a low clay content, a high sand content and a high density (Van Wallenburg and Markus 1971). The cover of “toemaak” is completely anthropogenic and contains many visible fragments from pottery, rubble, pipe bowls and other elements from household waste. Accordingly, this A1-horizon is characterised by high levels of heavy metals, like lead, copper and zinc (Lexmond et al. 1987). In Figure 3 the spatial distribution of “toemaakdek” in the central western part of the Netherlands is shown.

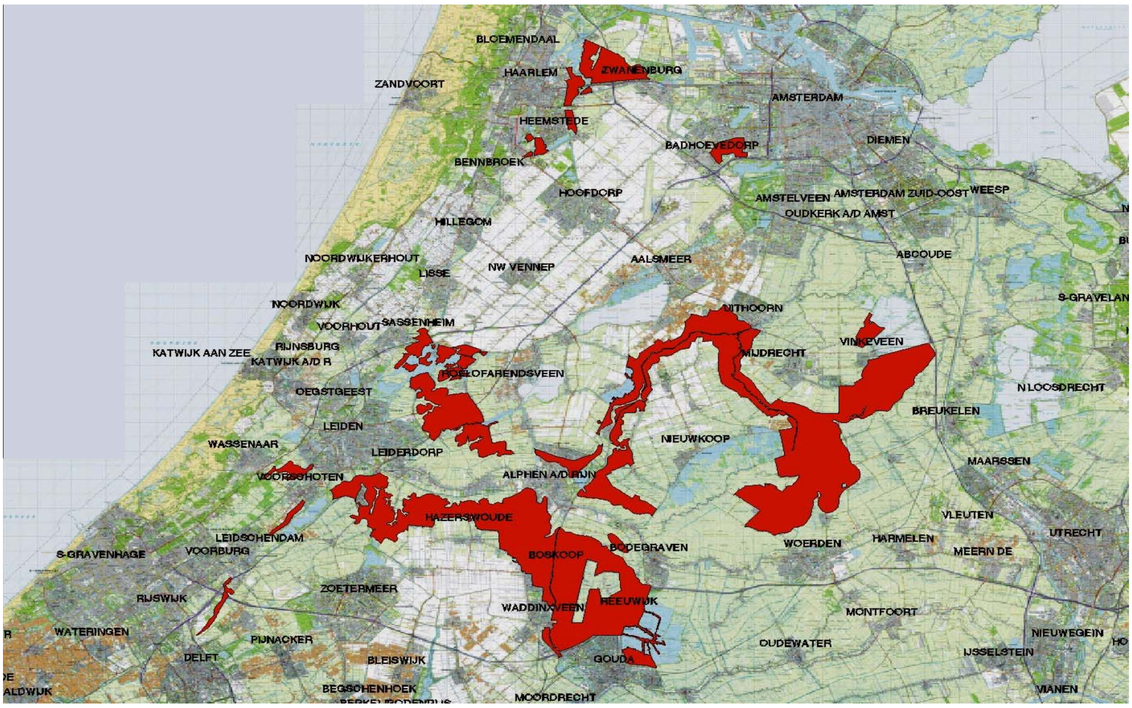

Figure 3. Spatial distribution of “toemaakdek” in the central-western part of the Netherlands. The presence of “toemaakdek” on the Dutch soil map is indicated in red. Urban regions are in grey. Typically, “toemaakdek” contains both increased diffuse contamination and physical rubble, like broken pottery and smokepipe heads.

Recently, we determined the concentrations of metals in samples from a few sections of the Demmerikse polder near Vinkeveen. The concentrations of lead, zinc, and copper were 678 ± 325, 313 ± 121, and 157 ± 86 mg metal/kg DW, respectively. The distribution of the metals in the field was patchy, with no clear systematic spatial gradient of metal concentrations. Furthermore, the distribution was not significantly different from a normal distribution (see Figure 4 for lead). The metal concentrations were concordant with former studies in this area (Bosveld et al. 2000; Chemielinco 1998; Lexmond et al. 1987; Tuinstra and Blok 1996).

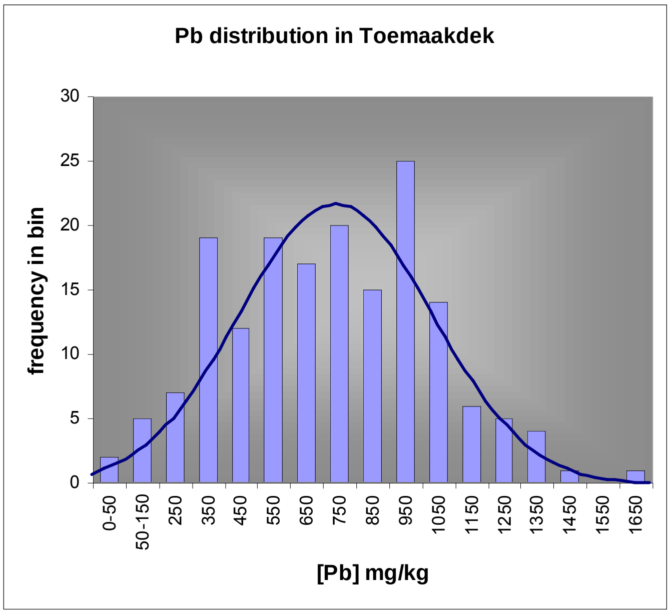

Figure 4. Distribution of lead in 174 samples from the Demmerikse polder (parcels 7 and 8 of the fields of Staatsbosbeheer). Samples were taken from the upper layer of the soil (5 – 10 cm depth), sieved over a 2 mm sieve, totally destructed and analysed by ICP-MS.

4.2 Research aims and approaches

In the framework of SSEO, several studies were conducted. For the sediments in the aquatic ecosystems, no clear contaminant gradients could be discovered, and only a few laboratory studies with biofilms from ditches in the Demmerikse Polder were conducted (Boivin et al. 2006b; Boivin et al. 2005). For the terrestrial ecosystems, small gradients were discovered in the area itself. However, the distribution of hot spots and clean places was unpredictable and patchy, and the concentrations of heavy metals in random samples followed a normal distribution (Figure 4). The use of less-contaminated reference sites outside the areas with “toemaakdek” was rejected on theoretical grounds, because the layer with “toemaakdek” is completely man made and unique.

Depending on the study, different tactics were used to obtain a relevant gradient for the demonstration of terrestrial ecosystem effects:

- Bacteria
  Large numbers of samples were randomly taken from the fields, typically about 100 to 150 per field campaign per day. Samples were also taken in conjunction with the Terrestrial Model Ecosystem studies (see below). Metal concentration in each sample was roughly determined with an XRF apparatus, first with fresh samples, and in a next step with dry and pulverized samples. Selection of samples for microbiological analysis was based on the results of the metal analysis. Depending on the type of experiment, between 4 and 30 samples were selected, demonstrating the highest gradients of lead, zinc and copper. Selected soil samples and soil extracts that contained bacteria were frozen and stored at -70 centigrade for later analysis.
- Nematodes
  In conjunction with the microbiological analysis, 26 fresh samples were selected for nematode analysis.

- Earthworms
  In one earlier study (Bosveld et al. 2000), the absence of gradients of contaminants on a sufficiently large spatial scale was acknowledged. In the SSEO programme, earthworm populations and potential metal effects were studied in association with field birds (see below).
- Terrestrial Model Ecosystems (TMEs)
  Large soil columns (width 17 cm; height 40 cm) were taken after mapping fields for metal concentrations (Kools 2006)
- Meadow birds
  No contaminant gradients at a relevant scale for meadow birds were available in De Ronde Venen. Instead, a distant reference site with peaty soil was chosen north of Amsterdam (Polder Zeevang) (Roodbergen and Klok Subm.).
- Geochemistry
  Geochemical processes related to sediments and metal contamination in peat areas were studied in enclosures in the field. These enclosures were manipulated.

4.3 Main results

A large number of soil samples were analyzed for soil characteristics and metal concentrations. Concentrations of lead, copper and zinc demonstrated a correlation. Also, metal concentrations in pore water, 0.01 M $CaCl_2$ extractions and complete soil samples demonstrated correlations. Surprisingly, total organic matter content negatively correlated to metal concentrations. It could be hypothesized that oxidation of peat decreased the organic matter content, and the bulk matter of the soil. This is indicative for subsiding surface levels in peat areas, an environmental issue of major concern in the Netherlands. However, this result is also indicative for an increased mobilization of heavy metals accompanying the oxidation of peat.

In Table 2, the concentrations of metals in several soil fractions are given. From a larger survey in the Demmerikse polder, about 50 % of all samples had total concentrations of Pb larger than the Intervention Value (about 700 mg / kg DW soil, after correction for the clay fraction and organic matter), and about 15 % of all samples had total concentrations of Cu higher than the Intervention Value (about 250 mg / kg DW soil).

Table 2. Concentrations of metals in three fractions of 20 samples of fields 7 and 8 of Staatsbosbeheer in the Demmerikse Polder. The mean ± standard deviation is given.

	total concentration (mg / kg DW soil)	pore water concentration (µg / l)	$CaCl_2$ extractable concentration (µg / kg DW soil)
Zn	387 ± 73	365 ± 283	5792 ± 4629
Pb	961 ± 325	12.4 ± 10.8	482 ± 475
Cu	216 ± 88	53 ± 22.7	1228 ± 611
Cd	1.45 ± 0.23	0.93 ± 0.53	57 ± 43

A team of three institutes carried out collaborative research with the aim of detecting field effects of metals in the Demmerikse polder. Boivin and Greve (Boivin 2005) looked at microbial communities in soil samples, Van der Wurff et al. (2006) looked at nematode communities, and Kools (2006) used terrestrial model ecosystems (TME). Their major findings were a range of strong indications of effects of metals on microbial communities, and to a smaller extent on nematode communities. Some isolated

observations indicating metal effects were obtained during research with soil columns in experiments with TMEs
.
Significant correlations were obtained between metal concentrations and shifts in the metabolic characteristics of microbial communities in soil samples from the Demmerikse polder, which could not be explained by other environmental parameters (see chapter on community-level physiological profiling elsewhere in this report). Also shifts in the structure of DNA molecules were correlated to metal concentrations. However, Pollution-Induced Community Tolerance (PICT) could not be observed unambiguously (see chapter on PICT elsewhere in this report), probably due to methodological problems, or the inability to measure PICT in indirectly metal-affected communities. Some correlations between metal-concentrations and shifts in the nematode community in soil samples of the Demmerikse polder were observed (unpublished data, Van der Wurff).

Van der Wurff et al. (2006) demonstrated a dependence of stress responses in TMEs on the field history of the soil column. For instance a heat shock had adverse effects on species richness in nematode communities in the highly contaminated soils, but not in the less contaminated soils. However, biomass density compensation occurred rapidly, i.e. tolerant species quickly replaced sensitive species, in all instances.

Various authors (Bosveld et al. 2000; Klok et al. 2006a; Kools 2006) found additional indications of metal effects in the Demmerikse polder. For instance, at highly contaminated spots, the percentage of juveniles in earthworm populations was increased, while total biomass was not affected. Furthermore, the biomass and community structure of enchytraeids was affected in the highly contaminated spots. Also, the degradation of straw was retarded in samples with high metal concentrations.

Considerable evidence was collected in favour of the hypothesis that metals had adverse effects on the terrestrial ecosystem in the Demmerikse polder, despite a large number of observations which did not clearly show these effects. For instance, the total biomass of many soil communities was not significantly lower in the highly contaminated samples. A highly unpredictable and patchy distribution of the contaminants, relatively short contaminant gradients, and variation in soil parameters disturbed clearly visible relationships between metal concentrations and field effects. The combination of positive results, however, suggests altered functional aspects in the terrestrial soil ecosystem, leading to a different stress response, altered metabolic characteristics of microbial communities, and shifts in community structure of bacteria and nematodes. Other effects of metals in the terrestrial ecosystem are plausible, for instance in earthworm communities and field birds. It can be hypothesized that dynamics and flow of elements and energy are altered due to the presence of metals in “toemaakdek”.

4.4 Summary of findings for the Ronde Venen research area

Large areas with peat in the western part of the Netherlands are covered with a unique layer of topsoil, the “toemaakdek”. Subtle effects of metals in this layer and the terrestrial ecosystem were demonstrated in the SSEO programme. Removal of “toemaakdek” is not an option for remediation, considering the long history of “toemaakdek”, the dimensions of the area (surface area and soil volume) and societal aspects. Besides, it was not shown that the level of effects should trigger remediation actions, according to the site-specific remediation thresholds in the current soil policy, except for exceedance of Intervention Values for some metals.
However, soil management will take place now and in the future. In an environmentally sound soil management of “toemaakdek” regions, the observations made by SSEO and others should be taken into

account. Soil management will affect the occurrence of adverse effects of metals on the terrestrial ecosystem in a positive or negative way. For instance, hydrological conditions are expected to have a large effect on the oxidation of peat, and subsequently on the mobilization of metals. The hypothesis is that a high ground water table will reduce the effects of metals in peat areas. Furthermore, depending on the type of nature development, the ecosystem will be more or less affected by metals. Key species and nature target types have different sensitivities to metals.

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5 Site characteristics and research at site "Afferdensche- en Deestsche Waarden" (ADW)

By: C. Klok

5.1 Site characterization and contamination

The floodplain Afferdensche and Deetsche Waarden (ADW), a small area of approximately 3 square kilometres, is situated near the village of Afferenden along the river Waal (longitude 51˚54'N, latitude 5˚39'E), one of the most important branches of the river Rhine (Figure 5).

Figure 5. The SSEO research site "De Afferdensche en Deestsche Waarden". Artists impression of the situation after implementation of the river management plan (source http://www.science.uva.nl/onderzoek/aot/adw).

The lowland part of the river Rhine has been changed over the last centuries from a meandering river with many shallow gullies and extensive floodplains into a river which is an important trade route for transportation of goods by ships. The main channel was deepened and embanked, and relatively small floodplains were squeezed between the channel and the embanking dikes. Following inundations, the sediment load transported by the river settled onto the floodplains. These sediments contain high amounts of pollutants such as heavy metals (Beurskens et al. 1993). The degree of contamination of the floodplains generally peaks in the soil layer at 0.5 to 2 m below ground level (Middelkoop et al. 2001; Middelkoop et al. 2002). The pollutant composition of the upper soil layer of the floodplains reflects the improved sediment quality, with moderate levels of cadmium and copper (2.8-5.7 mg/kg DW 62-126 mg/kg DW respectively) but still relatively high levels of zinc (514-980 mg/kg DW) (Klok et al. 2006b; Ma et al. 2004; Van Vliet et al. 2005; Zorn et al. 2005b). The soil composition itself is a typical floodplain soil consisting of large amounts of clay (9-30%) and moderate levels of organic matter (8 - 15%); pH values are rather high (7.3-7.4) (Klok et al. 2006b; Ma et al. 2004; Van Vliet et al. 2005;

Zorn et al. 2005b). Over the years sedimentation elevated the floodplains. To mitigate the risk of flooding of the inlands, the inhabited areas behind the dikes, the last were elevated resulting in the current situation that the river and floodplain area is situated above the inlands. After an extreme high water level in 1995, which led to the evacuation of 250.000 inhabitants, it was realized that continuation of the usual procedure of strengthening dikes to mitigate elevated floodplains cannot be continued over a long period. Alternative solutions to mitigate the risk of flooding of the inlands were formulated in the “Deltaplan” for the major rivers (www.ruimevoorderivier.nl), which was developed by the Dutch government. The ADW is a pilot project in this major river rehabilitation plan. A secondary channel will be created, implying the removal of parts of the minor embankments of the main river and a lowering of the floodplain area (Figure 6). This reconstruction coincides with an improvement of the nature function of floodplains. The area will become part of the Ecological Main Structure (in Dutch: EHS), which connects floodplains along the Rhine river with nature areas in the inlands.

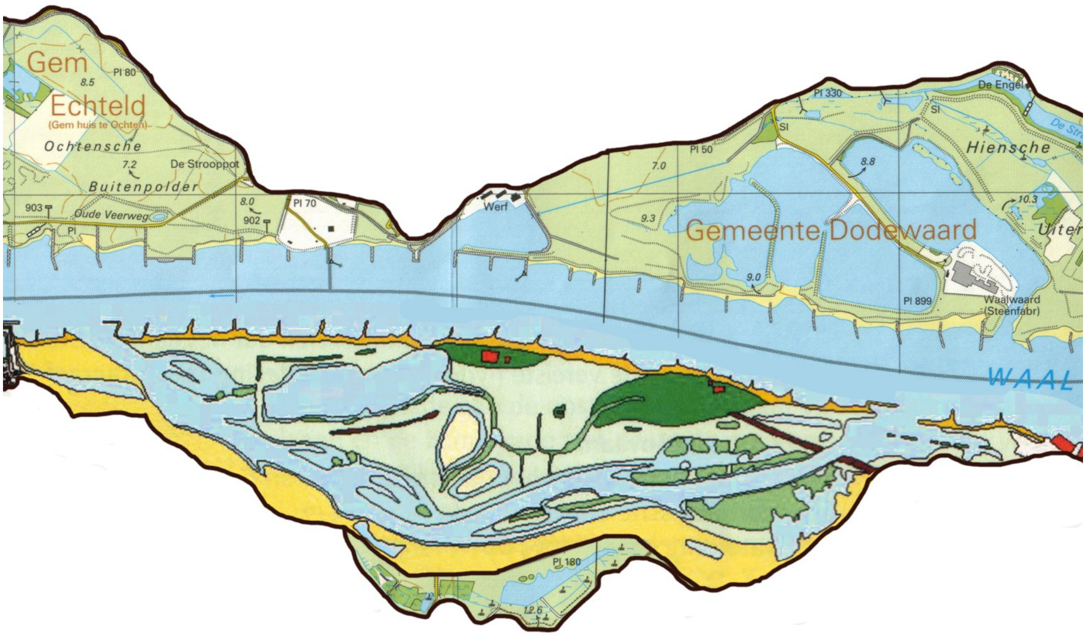


Figure 6. Illustration of the rehabilitation plan for the “De Afferdensche en Deestsche Waarden” (source http://www.science.uva.nl/onderzoek/aot/adw). Key issue is the new additional channel in the southern part of the area, which will increase the rivers’ discharge capacity and reduce flooding.

Given this new nature function of the floodplains, the main questions in the ADW area are:

1. What is the impact of the grey veil of pollutants on nature development?
2. How does flooding interfere with the possible effects of the grey veil of pollutants on species?
3. What is the role of bioturbation by organisms that live in floodplains on the availability of pollutants locked up in deeper soil layers?

5.2 Research spearheads and approaches

This section focuses on the terrestrial research in the ADW. Bioavailability was thoroughly studied in the ADW. Pollutant levels were monitored in earthworms (Ma et al. 2004; Van Vliet et al. 2005). The effects of pollutants on species composition, abundance and biomass of earthworms were studied (Ma et al. 2004) and the role of flooding was considered in both accumulation and effect studies. The single effect of flooding on earthworm population abundance and biomass was also studied (Zorn et al. 2005b), along with the abundance of small mammals (Wijnhoven et al. 2005). Floods not only deposit layers of sediments that are rich in nutrients and pollutants on floodplains, but the flooding itself also disturbs the biota in floodplains, resulting in an extensive drop in biomass and number of individuals (Zorn et al. 2005b). Flooding, therefore, induces extra variation in earthworm biomass and density, which may mask effects of pollutants and decrease the probability of identifying the effects of pollutants with a single study. To clarify the role of pollutants, a large study that included both SSEO floodplain sites and other floodplains, and in which inundation was included as an interacting factor, was conducted by Klok and Plum (in press). The role of bioturbation by earthworms was studied under standardized laboratory conditions (Zorn et al. 2005a) and in microcosms in the field (Zorn 2004).

5.3 Main results

The effect of pollutants on the bioavailability in earthworms showed enhanced concentrations of Cd, Cu, Pb and Zn (Van Vliet et al. 2005). Accumulation of heavy metals by earthworms proved to be species-specific and strongly influenced by flooding (Van Vliet et al. 2005). There are no clear effects of pollution on the species composition, abundance and biomass of earthworms (Ma et al. 2004). However, there was a strong response of earthworm biomass and abundance to flooding. Some earthworm species are even virtually absent when flood waters recede, whereas other species where strongly reduced in population number (Zorn et al. 2005b). Also, field characteristics such as location and elevation (which may point to lower risk of flooding) had a strong influence on earthworm biomass and abundance (Ma et al. 2004). Earthworm populations probably recover by re-growth from cocoons, which survive inundation. Inundation stress even results in adaptation of some species as exemplified by *Lumbricus rubellus*; it matures at a lower weight and at a corresponding younger age in frequently inundated floodplain sites, compared to sites which remain dry for longer periods (Klok et al. 2006b).

Small mammals showed a strong response in population abundance to floods. They recolonize the floodplains from refuges (areas such as heights and dikes that are not flooded) (Wijnhoven et al. 2006b). The availability of vegetation structure seemed to be very important in the process of recolonization, even guiding it (Wijnhoven et al. 2006b). However, small mammal densities at more than 30 m from the non-flooded areas were always lower than in non-flooded areas (Wijnhoven et al. 2005), which suggests that the colonization time between two successive floods (eight months) was not long enough for the entire recolonization of the ADW. Poor habitat connectivity, sparseness of non-flooded recolonization sources and small numbers of survivors lead to slow recolonization. Bioturbation by earthworms does result in large amounts of soil being transported from deeper layers to the soil surface. Bioturbation is a species-specific process depending on the soil layer in which earthworms live and their casting activity. Species that cast at the soil surface, like *Aporrectodea caliginosa* and *Lumbricus rubellus*, play the most important role (Zorn 2004). Under laboratory conditions the casting activity can amount to 2 kg/m^2 soil from the deeper layers to the surface (Zorn 2004). This casting activity can also make pollutants from deeper soil layers available to the local ecosystem.

5.4 Summary of findings for the ADW research area

Moderate levels of heavy metals were accumulated by earthworms and showed a species-specific response and a dependence on the timing of flooding (Van Vliet et al. 2005). Flooding has a major impact on the biomass and density of earthworms (Zorn et al. 2005b), and even resulted in a life-history adaptation: earthworms matured at an earlier age (Klok et al. 2006b). Also, the abundance of small mammals was strongly influenced by floods (Wijnhoven et al. 2006b). The effects of heavy metals on biomass and density on earthworms were unclear (Ma et al. 2004). Possible interactions of flooding and pollutants on biomass and density of earthworms cannot be excluded. Flooding induces an extra amount of variation in these parameters, on top of the effect of the pollutants. This implies that the probability to identify clear pollutant effects in a single study is low. Effects become apparent only if the number of replications is high enough, as was demonstrated by Klok et al. (2007). Recolonization of floodplains by small mammals is slow and may depend on landscape structures such as connectivity of shrubs (Wijnhoven et al. 2006b). Bioturbation by earthworms can indeed result in bringing pollutants, locked in deeper soil layers, to the soil surface (Zorn 2004; Zorn et al. 2005a).

6 Validation of ecotoxicological models

By: L. Posthuma, M. G. Vijver & F. Kuenen

In addition to the overview of observations on exposures and effects at the three study sites, the SSEO programme data imply that there is latitude for the validation of those models that play a role in current regulations. As mentioned in Section 1.1, studies of risks imply the application of models (in this case: ecotoxicological models), and validating those models is one of the targets of the research done for this report. Validation is, however, not always easy. This chapter addresses some major issues that are required to understand the validation studies presented in the next section.

6.1 Protection endpoints and measures for risks and effects

The relationships between environmental policies and the “true effects” of low-level diffuse pollution under field conditions are complex and can be summarized as follows (see Figure 7).

- At one end of the spectrum (left), there are policy targets. The chosen policy target (structural and functional protection) can often not be directly related to measures of effects in the field. In this case, in view of the choice to use a risk-based approach, the policy target has acquired the format of an operational measure of risk.
- At the other end of the spectrum (right), there are “true effects observed in the field”. However, these “true effects” are never attributable only to the exposure to a single compound (See Chapters 3, 4 and 5). There are always multiple compounds, and – moreover – other stressors and natural variability that shape local populations of species, or species assemblages. This means that operational methods are needed to identify which part of the “true response” is to be ascribed to pollution, and which to other causes.

Two intermediate steps are needed to link policy targets to effects, a requisite for model validation:

- A scientific analysis is commonly needed to propose an operational measure of risk, to quantify the target in measurable units (comparable to a “ruler”).
- Next, quantification of field effects on biota, and attributing them to the range of probable causes, is needed.

Both intermediate steps are needed to link policy targets to the field effects. On the risk ruler, limit values have been assigned in the past to discriminate between policy-unacceptable and policy-acceptable risk levels of exposure. Well-known examples of risk limits are the HC5 and the HC50 (Hazardous Concentration for 5 and 50 % of the species, respectively). These risk limits are used to discriminate between risks that are lower or higher than the Maximum Tolerable Risk (MTR) and are lower or higher than the trigger for remediation. The position of these risk limits should be such that in the ideal case the discrimination between unacceptable and acceptable *risks* is exactly linked to the policy target, namely the discrimination between acceptable and unacceptable *levels of effects*, via the risk ruler. In using this scheme, there are two roles: regulators define a protection endpoint (or an endpoint at which remediation is needed), and scientists (interfacing with the regulators) define a risk ruler that can be applied. Policy measures to prevent too much impact, or to trigger remediation, can be taken by using both the risk ruler (general, continuous) and the risk limits (specific cut-off levels). Good policy measures depend on the relationship between risk ruler and measures of true effects. Validation studies are concerned with the similarity of the risk and effect rulers, and are needed to confirm that the relationship between risk and effect rulers is good.

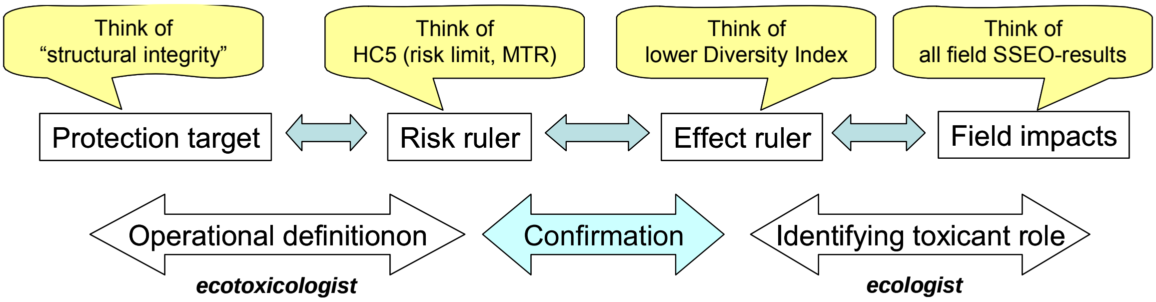


Figure 7. Stepwise linkage between policy problem, risk models, effect rulers (that quantify the role of toxic mixtures) and overall field effects observed along a pollution gradient, for example. On the one hand, for example, regulators target structural integrity and ecotoxicologists define a risk ruler to operationalize that target. For example, in the format of the Hazardous Concentration for 5% of the species, the 5%-criterion implies protection for the other 95% against any impact of exposure. On the other hand, ecologists must diagnose the role of toxicants from the natural variation found in the field. After diagnosis, they may have identified the relative contribution of toxic mixtures, or of specific compounds. Confirmation involves looking at both the risk ruler and the effect ruler. When both are related, this implies confirmation.

6.2 Limitations to validation

Validation needs be defined specifically, since there are various interpretations for this word – ranging from very broad to very narrow (Oreskes et al. 1994). In this report, we use a limited interpretation of this general concept: confirmation. Confirmation consists of the comparison of predicted values to observed values (e.g., predicted exposure to observed body residues in organisms), where the models to generate the prediction do not fully capture all mechanistic processes.

Confirmation has been defined by Oreskes et al. (1994). According to logical interference, many models in environmental assessments can neither be verified (its full "truth" has been demonstrated, meaning full reliability in any case), nor validated (it has a logically valid construction and, more trivially, its computer codes contain no known or detectable flaw). The former would require a theoretically impossible model structure and study design, the latter specifically points at uncertainties that remain in the input data and the data processing that cannot be fully solved by further studies. What we tried to do in this paper is to address the validity problem with the most feasible option, i.e. seeking confirmation. In accordance with confirmation theory, the greater the number and diversity of confirming observations (modelling results match with empirical observations), the more probable it is that the model is not flawed, and the more strongly the model is confirmed. In serious cases, mismatching can cause the rejection of the whole model. It can also initiate model adaptation when logical omissions or inappropriate input data are identified. Finally, it can lead to the setting of limitations on model application if the model is useful for a certain application, but not for others. In short, the consequence of confirmation studies for model use can be the following: to adopt, adapt or abandon the use of the model. In the present study, the findings of the confirmation studies are used to draw conclusions on model use in regulatory practice.

6.3 The risk assessment paradigm and exposure and effect validation

Basic to risk assessment modelling is the risk assessment paradigm (Figure 8). A risk assessment always consists of an exposure assessment (quantifying exposure of biota), an effects assessment (quantifying the sensitivity of exposed biota) and a risk characterization. The latter merges the exposure data with the sensitivity data (the exposure and the effects assessments), which results in a description of risk (risk characterization). Regarding the issue of validation (more specifically: confirmation), this implies that validation studies can focus both on exposure issues (by comparing predicted exposure with observed uptake) and on effects issues.

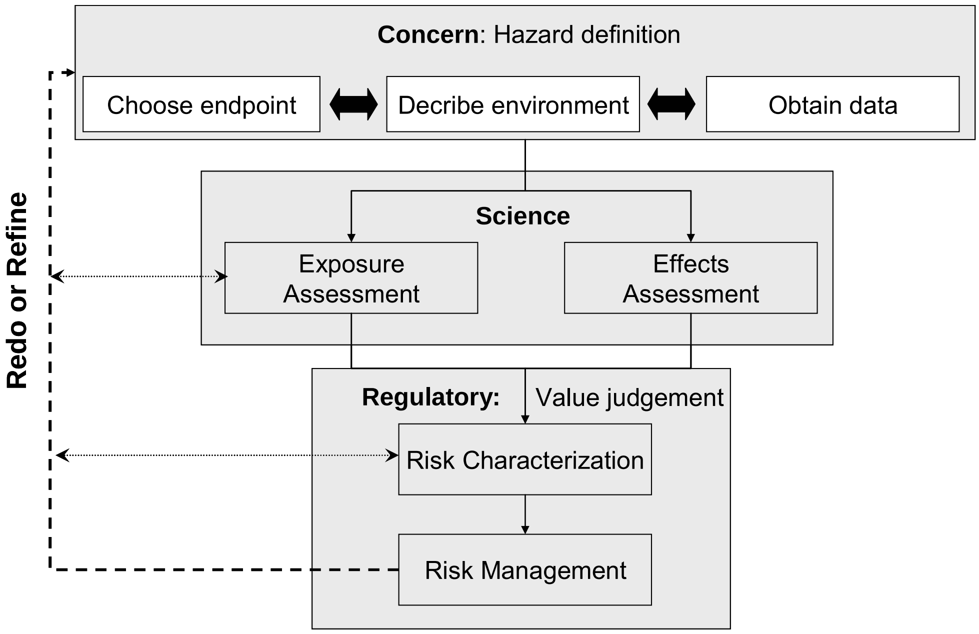


Figure 8. Scheme of the internationally adopted risk assessment paradigm. When a *concern* is identified, the protection or assessment endpoint is defined (e.g., "structural integrity of ecosystems") relevant data are collected, and *scientific analyses* are made of exposure and effects (the latter from dose-effect knowledge). These are combined in a risk quantity (probability and magnitude of likely effects) and interpreted for *regulatory purposes* by applying a value judgement, such as the choice of a criterion for Maximum Tolerable Risk. When the risks are considered too high, the assessment can be refined and/or risk management actions can be taken to reduce risk (e.g., by lowering probability of exposure).

6.4 General validation approaches

The general outline of the set of validation studies in this report consists of two working hypotheses (see Figure 9):

- The testing of one model in different case studies (different sets of field data), so as to consider model robustness: does the model perform well in different circumstances, and if not – why not?
- The testing of various models on one case study (one field site), so as to consider differences between models in prediction accuracy, and considering the relative performance of models:

which model is of sufficient precision for a given case, and can this model be fairly simple (for reasons of cost-effectiveness under practical use conditions)?

Together, the set of studies should yield information on the validation status of the different models. This should result in appropriate use of models. Note that testing the general validity of the working hypotheses would require a larger set of studies than is available from the SSEO study sites. Original data were compiled to obtain the data for the validation studies. This was done in collaboration with the researcher(s) who designed and executed the different studies. Each validation chapter is thus based on one original scientific manuscript, or a combination of related manuscripts, and is written by a primary author who was involved in the pertinent study as well as in this validation project.

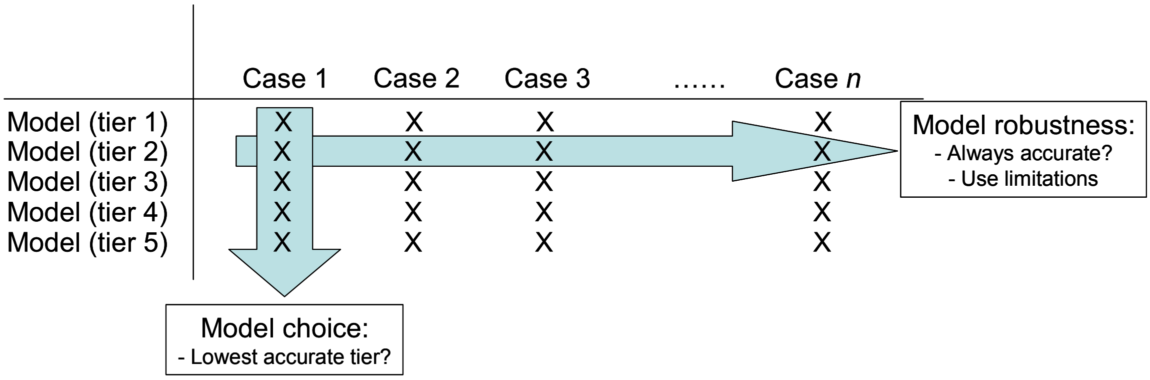


Figure 9. The working hypotheses of the validation studies: (1) when each case study is analysed by a single model, conclusions can be drawn on model robustness, i.e. the ability of a model to accurately predict field phenomena in multiple cases; (2) when one case study is analysed by various models, such as a statistics-based Species Sensitivity Distribution and an ecology-based food-web model, conclusions can be drawn on the model that predicts the field phenomena, with sufficient accuracy; that is, which tier of modelling is needed for a given assessment problem.

6.5 Selection of models for incorporation in this report

At the start of the SSEO programme, study sites and compounds were selected such that an array of ecotoxicological models could be the subject of validation studies. The models that could play a role in this validation effort have been in use to give a scientific underpinning to various existing environmental policies. They were selected for the current research, *because* they were practically used, and mainly not for other reasons (e.g. a scientifically preferred modelling approach). The models that were the subject of the present study are listed and explained in the report by Posthuma et al. (2005). The models focus on statistical analysis of ecotoxicological test data (such as the ETX and OMEGA123 models), some mechanism-based models (like OMEGA45 and PODYRAS) and some expert models (for example PERPEST). For further information see the previous report. The chapters on validation in this report also give a short introduction to each of the models.

6.6 Selection of studies for incorporation in this report

The studies that are considered in this report have been selected from a set of more than one hundred papers from SSEO researchers. The selection of studies primarily follows from the criteria already formulated at the start of the SSEO programme, focusing on three SSEO study sites, and the compound mixtures that are characteristic for those sites. These sites were chosen since they represent frequently encountered cases of diffuse pollution in the Netherlands. Furthermore, the study selection was done according to the following principles:

1. The study pertains to a validation study of either of the models mentioned in the inventory study on model validation (Posthuma et al. 2005); this implies that emphasis is on the contributions from researchers involved in the project (Posthuma et al. 2001); the study data were obtained from one of the three study sites from the SSEO programme
2. The study addresses some broader perspectives on results obtained in the SSEO programme (without detailing on all 100+ studies)
3. The study addresses some perspectives obtained from ongoing research of the research groups involved in the project to broaden the scope and to avoid unjustified generalisations based on the three SSEO sites only.

Due to these choices, the present report has a limited scope, namely that of the set of models chosen for the validation studies, the SSEO studies and only few additional studies. Hence, this report was *not* intended to be, and is *not* a formal scientific review of, the entire SSEO programme. The studies focused both on exposure and effect studies.

6.7 Overview of chapter contents

6.7.1 Exposure and validation of exposure models

In the section of this report on validation (Section 2), we first address exposure issues. In a set of chapters, various aspects of exposure assessment are considered.

- In Chapter 7, “Natural background and anthropogenic enrichment”, attention is given to the role of natural background concentrations and anthropogenic enrichments in the causation of impacts.
- In Chapter 8, “Chemical availability for uptake”, attention is given to the physico-chemical drivers that determine that availability of compounds for uptake. The motive for this study is that uptake of compounds from the environment is often hypothesized to be dependent on local sorption characteristics of the soil or sediment substrate.
- In Chapter 9, “Modelling the solid-solution partitioning of heavy metals and arsenic in embanked floodplain soils of the Rhine and Meuse” this issue is addressed too.
- In Chapter 10, “Implementation of “black carbon” correction factors in bioavailability and food chain accumulation models for hydrophobic organic compounds” the focus is, similarly, on organic compounds and the major role of black carbon in determining exposure of biota.

Although the emphasis of the SSEO programme is on effects of the diffuse exposure to contaminants, risks cannot be appropriately assessed without considering the influence of habitat characteristics on exposure (see Figure 8).

Where the aforementioned chapters address the *abiotic* aspects of the bioavailability concept, we continue on the path of exposure assessment, but now add biotic elements (bio-availability consists of both physico-chemical drivers and species-dependent uptake characteristics, see Dickson et al. (1994)).

- In Chapter 11, “Metal accumulation in the earthworm *Lumbricus rubellus*: model predictions compared to field data”, attention is given to the direct uptake of compounds from the substrate in a species, in this case a worm species.
- In Chapter 12, “Modelling cadmium accumulation in a herbivorous and carnivorous terrestrial food chain” attention is given to species that are exposed via other species, in this case different rodents, via exposure of their food. In this paper, two food chains are described. In these Chapters, the OMEGA model is used to analyse the data.

Continuing on the theme of exposure as major object of study, but now also involving effects assessments, there are two relevant chapters:

- In Chapter 13, “Population growth and development of the earthworm *Lumbricus rubellus* in a polluted field soil, and possible consequences” the theme of food chains is addressed, regarding earthworm exposure and effects and the possible effects on the godwit, higher in the food chain.
- In Chapter 14, “Food web modelling of toxicant exposure and addressing functional groups” the theme is worked out further conceptually, according to the food web approach, thus concerning multiple-species exposure as well as multiple-species sensitivity modelling.

6.7.2 Effects and validation of effect models

Studies on field effects of contaminants are relevant for validation of effect-oriented models. In comparison to the validation studies of exposure models, the validation of effect-related models is more complicated. This relates to various problems:

- First, although the predicted effect usually follows from the local concentrations and the model structure, the modelling may involve both a direct assessment of effects based on total concentrations and an indirect one, whereby the effect modelling is combined with an exposure model. In the latter case, the net prediction of exposure and effect models is compared to field data;
- Second, there are various types of effects that are predicted. They range from community-level probabilistic predictions (identifying the fraction of species that is probably affected by the exposure), to population or foodweb models that are founded in the biology of the species and the characteristics of foodwebs, respectively;
- Third, the biotic phenomena that are observed in the field can theoretically be a product of the exposure situation only, but – more commonly – these phenomena are the result of an array of stress factors. This implies that – for validation – the effects that result from exposure to toxic mixtures should be separated from the effects of the other stressors.

In a set of chapters, various aspects of effect-model validation are addressed.

- In Chapter 15 “Predictive value of Species Sensitivity Distributions for effects of pesticides in freshwater ecosystems”, a study is made of the accuracy of the Species Sensitivity Distributions (SSD) concept in predicting field effects.
- In Chapter 16, “Impact of TriPhenylTin-Acetate (TPT-Ac) in microcosms simulating floodplain lakes: comparison of species sensitivity distributions between lab and microcosm” this theme is worked out further.
- In Chapter 17, “Using the expert model PERPEST to translate measured and predicted pesticide exposure data into ecological risks”, the PERPEST model is described and tested. Observed responses in articificially exposed ecosystems have been used to create a database, which is subsequently the basis for a predictive model.
- In Chapters 18, “Detecting effects of “the grey veil” of pollutants on earthworm populations in river floodplains. Does flooding blur the picture?”, 19, “Do earthworms (*Lumbricus rubellus*) adapt to flooding in wetlands by early maturation? Support from field data”, 20, “Combined effects of pollutants and inundation stress on earthworm populations in river floodplains”, and 21, “Impact of climate change and floodplain rehabilitation on terrestrial earthworm food chains”, the attention is focused on population-level effects, in this case on earthworm field populations. By applying population modelling and energy-budget modelling, these chapters unravel the role of toxicant exposure and inundation frequency in shaping the composition of field populations. The models used are PODYRAS (as introduced in the model inventory report) and the Dynamic Energy Budget (DEB) model, described by Kooijman (2000). These analyses address the aspect of multiple stressors influencing field biota, in this case populations.

- In Chapter 22, "Impact of the "grey veil" of sediment-bound contaminants on macro-invertebrate communities in the Rhine-Meuse delta" the attention is focused on the aquatic compartment, and a set of studies on the field effects of contaminants is described, showing an array of possible responses to mixtures of contaminants and other stressors.

6.7.3 Additional studies, without validation of models

In an additional set of chapters, attention is given to the issue of demonstrating toxicant responses, or the lack of such responses, in a field situation and the associated problems regarding some specific issues:

- In Chapter 23, "Effects of heavy metals on the structure and functioning of detritivores in the contaminated floodplain area in the Biesbosch, the Netherlands", it is described how low bioavailability of compounds implies low effects.
- in Chapter 24, "Metals affect secondary stress sensitivity of nematode field populations", it is described how responses may be covered rather than overt (latent stress sensitivity difference between exposed and non-exposed communities).
- In Chapter 25, "Pollution-Induced Community Tolerance as an ecotoxicological model to demonstrate effects?" an analysis is made of the occurrence of Pollution-Induced Community Tolerance (PICT) as a measure of stress on communities in the field.
- In Chapter 26, "Analysis of compiled microbial functional traits" the focus on ecosystem functional characteristics is worked out further, shedding light on some current methods to quantify functional traits of microbial communities, as well as the possible divergence between communities as a consequence of toxicant exposure.
- In Chapter 27, "Spatial aspects in ecotoxicology: heavy metal accumulation risks in diffusely and moderately polluted floodplains" the attention is focused on an aspect that is rather new as compared to classical protective risk assessments: the factor of spatial distribution of exposure and species distribution.

Apart from the studies done at the three SSEO study areas, further insights into the role of diffuse contamination in influencing field populations of particular species and in shaping local species assemblages were obtained by analysing monitoring data:

- In Chapter 28, "Toxic effects, multiple stress and butterfly abundance trends in a nature area in the Netherlands" a case study is presented from a case outside the SSEO programme, providing a case where effects were detected, and were (most probably) attributable to exposure to toxicant mixtures. The case study concerns the decline of butterfly populations in a Dutch nature reserve. Based on the combination of the monitoring data with ecological characteristics of the studied species and ecotoxicological modeling, an explanation was sought for an observed decline of butterfly populations. The ecotoxicity model that was used is the SSD model (see model inventory report (Posthuma et al. 2005)).
- in Chapter 29, "Analysing data from monitoring networks: the role of toxic compounds and other stressors in shaping natural assemblages of fish species", a case study on the data analysis of a large-scale monitoring of river systems in Ohio, USA is described. This study is also an extension of the SSEO programme. Again, based on the combination of the monitoring data with ecological characteristics of the studied species and ecotoxicological modeling, an explanation was sought for the observed patterns in fish species compositions across sampling sites. This analysis considered the role of toxicant mixtures relative to a set of other potentially stressful water variables.

Given the set of analyses made in the previous chapters, in the final Section (5) of this report an attempt is made to integrate all findings. This is done in order to provide a science-based set of possible consequences for the various regulations in which toxic compounds play a role. This concerns the source-oriented regulation of toxic compounds, the compartment-oriented management of soil, sediment and water, and the species- or nature-oriented regulations.

Section 2. SSEO-model validation studies

The following set of chapters is related mainly to the issue of validation of models that have been used – and are still used – in a regulatory setting. These models were selected and described in a preliminary study (Posthuma et al. 2005). The validation studies were the key part of the research work that was done for the current report, in accordance with the project description for the funding by NWO (Posthuma et al. 2001); this was included in the SSEO programme description (NWO 1999). Hence, this section concerns the studies in the SSEO programme in which modelling was applied as part of the approach to describe and understand the phenomena that were observed in the field.

7 Natural background and anthropogenic enrichment

By: M.G. Vijver, J. Spijker, J. Vink & L. Posthuma

7.1 Problem definition

Various compounds, especially metals, have two possible origins: the natural background in the geological formations, which determines the basic quality of soils, sediments or water bodies, and the enrichments from anthropogenic activities. Whereas the latter are a possible subject of clean-up activities or emission control, the former is a fact of life. They may be the subject of risk management (natural backgrounds may imply risks, like the naturally arsenic-rich seepage water in some Dutch polders that affects cattle health), but still they may be only incidentally subject to clean-up operation planning.

7.2 Approach

So-called baseline concentration data of metals (Cd, Cu, Pb, Zn), which concern the natural background concentrations of typical Rhine-delta sediments, were collected from sediment layers deposited before 1850 AD. A baseline model was fitted to these data, using one Al proxy. In fact, aluminium is known as a stable composite of clay-mineral deposits, and strongly correlates with the metal content of undisturbed sediments. While the clay content of a sediment layer can be variable, the anthropogenic enrichment can be calculated by acknowledging the natural association between metal and aluminium contents by applying a baseline model. When this geogenic baseline model is available, the anthropogenic enrichment of an environmental sample can be determined by subtracting the natural background (derived from the baseline) from the measured total concentration of a compound in any environmental sample. These models are described in Vijver et al. (in prep.).

7.3 Main results

An example of the difference between a geogenic baseline and measured top-layer sediment metals concentration of is shown in Figure 10.

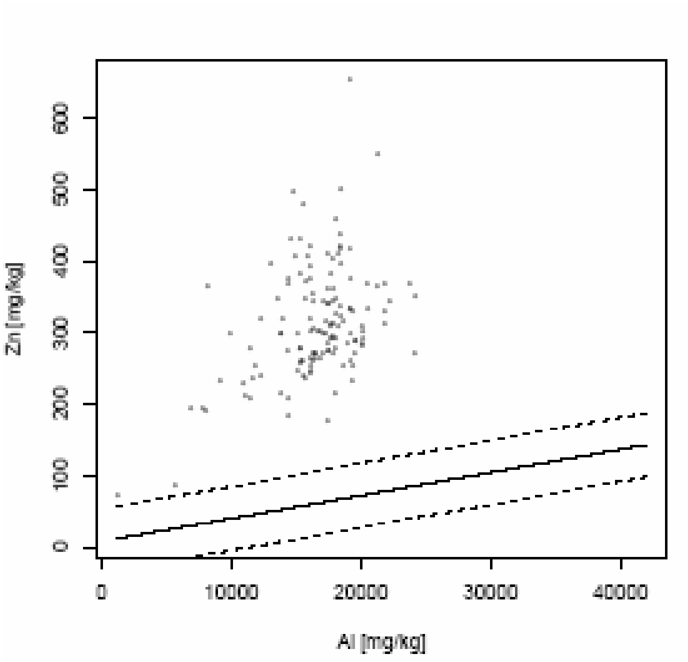


Figure 10. Geogenic concentration data of zinc (sediments deposited before 1850 AD), the baseline model derived (black line, model error shown by dashed lines, 2.5 times error scale) as a function of the aluminium content of the sediment, and present-day sediment concentrations of zinc in top-layer sediments of the Waal river (dotted line).

7.4 Implication and possible use

Many risk assessment protocols do not handle the issue of natural backgrounds. This implies that the risk attributed to human activities now and in the past can be overestimated, which can in turn trigger remedial action. For metals, regulatory and scientific concerns have been expressed about the fact that the definition of clean soils on the basis of the target value was problematic since the current concentrations in soil systems remote from any point source often contain metal concentrations that exceed that target value. In other words, the concern arose that there was hardly any soil in the Netherlands that would fit the criterion for “clean soil”. As a consequence, the Added Risk Approach was defined (Struijs et al. 1997), in which the natural background concentration could be taken into account. Practical risk assessments could profit from addressing natural background concentrations by using the baseline model approach. This will result in an improved risk assessment conclusion in cases where such backgrounds are present, and therefore a better risk management for those situations. Redundant actions will be averted.

rivm

8 Chemical availability for uptake

By: M.G. Vijver, J. Spijker, J. Vink & L. Posthuma

8.1 Problem definition

Depending on chemical characteristics as well as substrate characteristics, the availability of chemical compounds for true uptake in exposed organisms may vary by orders of magnitude between various compound-substrate combinations. Evidently, since risk is a function of both exposure and sensitivity, the issue of “bioavailability” is an important concept for risk assessment. Risks can be highly overestimated or underestimated if the data sets used to calibrate the risk assessment model consist of situations biased towards higher or lower chemical sorption as compared to the field situation. Bioavailability is a complex concept that can be broken down into at least three distinct phases (e.g., Dickson et al. 1994):

- environmental availability (physico-chemical “supply”),
- environmental bioavailability (the biological “demand” of a species), and
- toxicological availability (the availability at the site of action within the tissue or an organism).

All these phases are dynamic, and there is both a site (abiotic substrate) and a species-specific (biotic) component in this definition.

Bioavailability issues are usually addressed from the viewpoint of supply, by both modelling and measurement. When the supply is addressed by measurements, environmental samples are usually extracted by shaking in a fluid extractant, and determining dissolved and total concentrations as well as of their ratio (partitioning coefficients). Common examples are hot or cold acid extractions, or extraction in weak salt solutions. Models are applied too, to determine the appearance forms of a chemical compound. For metals, there is an array of metal species recognizable in a sample, whereby the fraction of each species is determined by the substrate composition. When both extraction and enrichment data are available (see Chapter 7), it can be investigated whether an extractable fraction is correlated to the enrichment. In this way, various techniques can be compared and used in practice as appropriate. For example, in prospective risk assessment (“what-if” problem definitions) it is impossible to use extraction-based methods, and modelling is the only solution. In such cases, it is good to be able to link model results to other approaches. The study is described in detail in Vijver et al. (In prep.).

8.2 Approach

Measured solid phase (total) and pore water (dissolved) concentrations of metals, as well as various substrate and pore water characteristics, were analysed for sediments collected in the floodplains of the Waal river. For these samples, enrichment data were also obtained using the baseline approach (see Chapter 7). The extractable fractions were determined for various extraction fluids.

8.3 Main results

8.3.1 Extractions and enrichments

The comparison between extraction data and enrichment data suggested that dissolved concentrations obtained by cold acid extraction with 2 M HNO_3 were associated best to the anthropogenic enrichment. The analyses supporting this conclusion are shown in Figure 11.

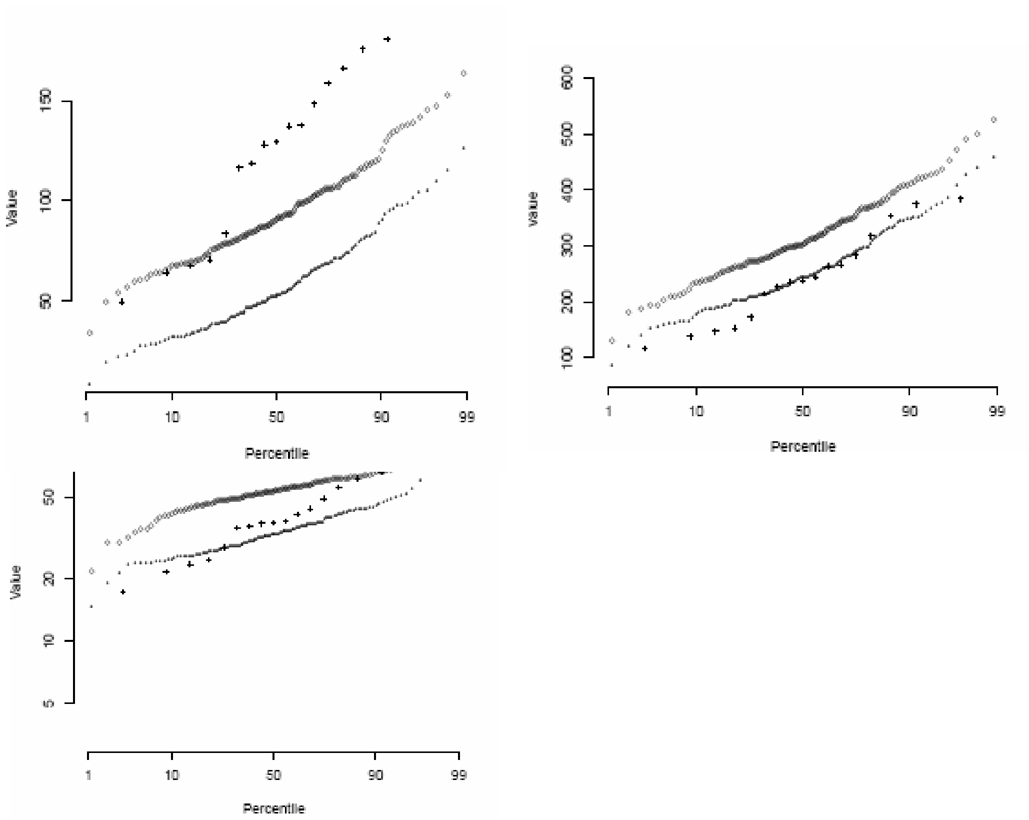


Figure 11. Relationships between extracted fractions (cold and warm acid extraction) and calculated enrichments in top-level sediments of the Waal river. The plots show the cumulative frequencies of all subsets of values for anthropogenic enrichments (triangles) and hot-measured (circles) and cold-measured (plusses) 2M HNO_3 soil extracts.

8.3.2 Soluble fractions and models predicting those fractions

Measured soluble fractions were compared to predictions made by various models: WHAM VI (model 1), floodplain-specific partitioning functions derived from a training set of Dutch river floodplain partitioning data (model 2), and a mechanistic-kinetic model (BioChem-Orchestra, model 3). The former two models were fitted to all data collected for his study, the latter only to three cases. In the latter model, a relationship between soluble concentrations and depth can be derived (Figure 12). These findings corresponded well with measured pore water concentrations.

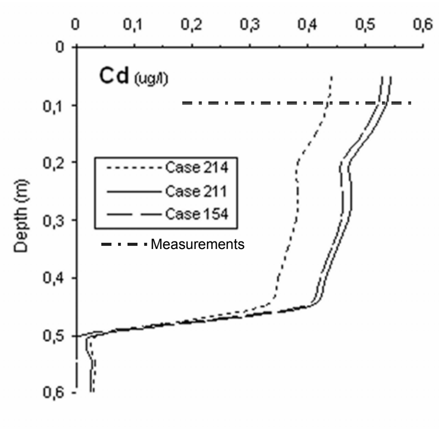


Figure 12. Illustration of three BioChem-Orchestra scenario analyses of pore water concentrations of cadmium based on measured total Cd concentrations, the mineralogical composition of the soil and the local redox conditions of the sites, including variation with depth (pH=7.3 constant with depth; [PO4]=0.02 mol/kg; [SO4]=0.01 mol/l; water level at –0.2 m). The range of measured concentrations is shown by the horizontal dotted line (3 measurements).

The relationship between predicted and observed concentrations for the various models that can be applied to predict dissolved fractions is given in Figure 13. This figure shows that various models, based on different sets of basic principles and approaches, are different with respect to their accuracy in predicting dissolved exposure concentrations.

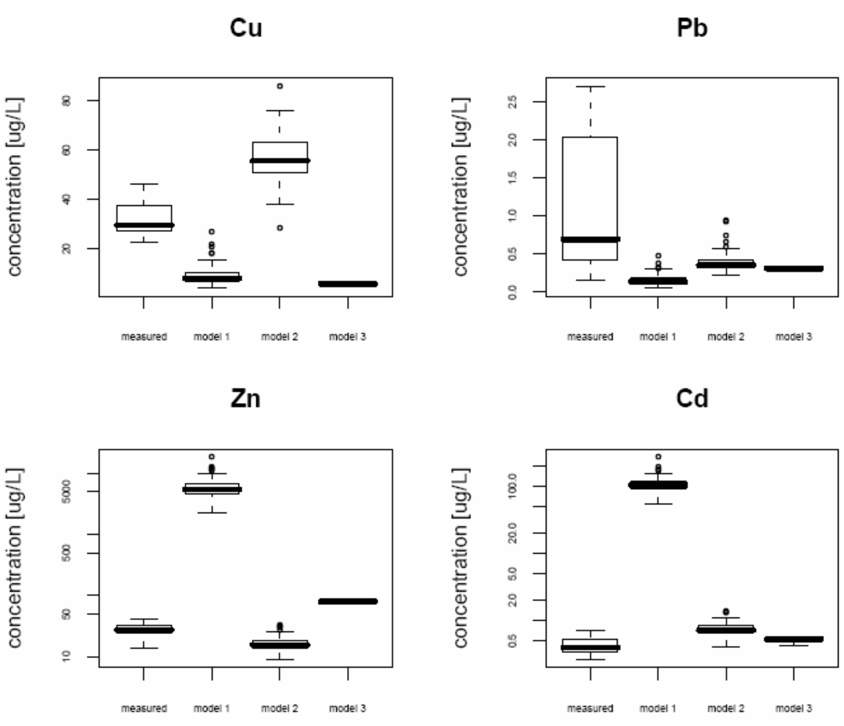


Figure 13. Comparison of pore water concentrations measured in the field (first box plot) and calculated with WHAM (model 1) using the calculated enrichment, calculated using functions specific for floodplain systems (model 2), and calculated using the kinetic model (model 3).

For copper, model 2 predicts best, but all model predictions are relatively close to the observed dissolved fractions. For lead, the variability of the field-based measurements exceed the variability of the predicted concentrations by far, but the median estimate of all models is a slight underestimation of the median observed value. For zinc and cadmium, model 1 performed worst; this model over-estimated the dissolved fraction (exposure), while the other two models performed reasonably (showing slight over- or underestimations of observed dissolved fractions). The over-estimation is related to the missing issue in this model – accounting for precipitation.

8.4 Implication and possible use

“Bioavailability” analyses are an integral part of risk assessment (see Figure 8), and are often considered very important, due to the quantitative importance of sorption of compounds to the matrix. Hence, appropriate exposure modelling is crucial for appropriate risk characterization. The current analyses show that both measurements and modelling can be of help in determining the dissolved fraction, and – in turn – this is considered important, as it is assumed that exposure is often mediated by pore water transfer of compounds. Both measurements (extractions) and modelling can provide insights into the locally dissolved fraction. The comparative analyses show, however, that the basic principles and approaches of a model influence the output, i.e. when neglecting precipitation in the model, the dissolved fraction can (by definition) be over-estimated (specifically for cases where precipitation occurs). On the one hand, this shows that modelling may yield inaccurate results (they overestimate exposure). However, since the risk paradigm (Figure 8) starts with the problem definition, one can consider this “flaw” of overestimation to be acceptable in cases where the problem definition has a conservative context. For example, for setting safe quality criteria, the regulatory context is “conservative” (the criterion should be safe even in worst case conditions). In such cases, over-estimation of exposure may be a “desired feature”, comparable with the introduction of safety factors. A consistent ranking of the models, from always simple and conservative to more complex and precise, could not be made, given the limited set of compounds studied, and their individual physico-chemical behaviour in the environment. However, the impression exists that the mechanism-based modelling (Orchestra) is indeed more precise than others in most cases, and it can also address scenario problems. The latter are important when considering large-scale river management incentives, where the physico-chemical conditions in the river sediments will change.

9 Modelling the solid-solution partitioning of heavy metals and arsenic in embanked floodplain soils of the Rhine and Meuse rivers

By: M.G. Vijver & J. Vink

9.1 Problem definition

Mechanistic geochemical modelling was compared with a statistical approach. Having a simpler model in the analyses of field situations could be faster, could require less data and could be cheaper than obtaining full mechanistic geochemical modelling, but could yield less accurate results. Depending on the policy question, simple and fast answers are, however, sometimes preferred above detailed information. Within the study described in detail by Schröder et al. (2005), solid-solution partitioning data of heavy metals in floodplain soils were analysed. Statistical relationships were derived based on field measurements, and the prediction of these relationships were compared to a more complex mechanistic geochemical model in the present study.

9.2 Approach

To characterize the heavy metal contamination of embanked floodplain soils in the Netherlands, 194 soil samples were collected at 133 sites located in the Dutch territorial part of the Rhine and Meuse river systems. Total amounts of As, Cd, Cr, Cu, Ni, Pb, and Zn were measured in the soil samples and the metal fraction extractable by 2.5 mM $CaCl_2$. Statistical relationships were derived based on field measurements. The predictions generated by these statistical relationships were validated in other floodplain systems and outcomes on metal partitioning were compared to the results as predicted by a more complex mechanistic geochemical model.

9.3 Results

A strong correlation was found between heavy metal contamination and organic matter content, which was almost identical for both river systems. Speciation calculations by a fully parameterized model showed the strengths and weaknesses of the mechanistic approach (for a discussion, see Schröder et al. (2005)). Cu and Cd concentrations were predicted within one log scale, whereas adjustments of some model parameters were needed for Zn and Pb. The statistical fitting approach produced better results, but is limited with regard to the understanding it provides. The log-transformed RMSE (root mean square error) for this approach varied between 0.2 and 0.32 for the various metals. Table 3 shows the outcomes of the predictions by the log-RMSE values generated by using the geochemical model for Cd, Cu, Zn, Pb, and As with a reduced number of input variables.

log RMSE	Cd	Cu	Zn	Pb	As
fully parameterized model	0.523	0.268	1.298 (1.245)	2.765	0.656 (0.395)
modified model	0.513	0.272	0.697 (0.517)	0.45	0.740 (0.508)
average P(tot), Mn (tot) and Al (tot)	0.513	0.272	0.697 (0.517)	0.452	0.740 (0.507)
average Fe(tot), P (tot), Mn (tot) and Al (tot)	0.513	0.273	0.697 (0.517)	0.577	0.775 (0.594)
average P (tot), Mn (tot) and Al (tot), DOC from SOC	0.499	0.269	0.696 (0.517)	0.451	0.740 (0.503)
average P (tot), Mn (tot) and Al (tot), DOC from SOC, pH from Ca (tot)	0.503	0.269	0.692 (0.629)	0.419	0.659 (0.543)

Table 3. Root mean square error values for various models applied to a data set on metals in sediments. The RMSE values are calculated from the log-transformed concentrations. Tot = total concentration after Aqua Regia destruction. Values between brackets are samples with pH>6.8

As can be seen from the results, the fully parameterized model does predict in most cases better than the simplified, modified models. Nevertheless, predictability in most cases decreases slightly with the extra data input.

9.4 Implications and possible use

The fully parameterized model predicts solid-solution partitioning of heavy metals in oxic floodplain soils. The statistical approach resulted in even better and more accurate predictions for all metals in a 2.5 mM $CaCl_2$ extract. Although both methods have their advantages, this statistical approach can be used with a reduced set of variables, and the equations can be easily applied to calculate the availability of heavy metals in river flood plain soils in the Netherlands under oxic conditions. The careful modelling of speciation and adsorption processes is a useful tool for the investigation and understanding of metal availability in floodplain soils.

10 Implementation of “black carbon” correction factors in bioavailability and food chain accumulation models for hydrophobic organic compounds

By: C.T.A. Moermond, M. Hauck, J.J.G. Zwolsman, A.J. Hendriks, T.P. Traas & A.A. Koelmans

10.1 Problem definition

Mobility, bioavailability and bioaccumulation behaviour of Persistent Organic Pollutants (POPs), such as polychlorobiphenyls (PCBs), polyaromatic hydrocarbons (PAHs), brominated flame retardants, hydrophobic pesticides or dioxins, appears to be limited by the presence of black carbon (BC) in sediment. Due to strong sorption to BC, dissolved concentrations of POPs in water are lower than those predicted by traditional equilibrium partitioning. To date, this limitation has not been accounted for in risk assessment procedures, which may imply that risks of POPs are seriously overestimated. Hence, the question is to what extent BC (soots, chars) may limit the bioavailability in soils and sediments in the Netherlands, and how this limitation should be modelled (Koelmans et al. 2006).

10.2 Approach

The effect of BC on POP accumulation was investigated in three parallel SSEO studies. All of them combined empirical data analysis with accumulation modelling.

1. *Modelling the effect of BC on steady state BSAFs for POPs in benthic macro-invertebrates.* Field-based PCB and PAH biota-to-sediment accumulation factors (BSAF) for benthic invertebrates were determined in different seasons in chemically similar, but ecologically different, floodplain lakes (fish-dominated turbid, algae-dominated turbid, and macrophyte-dominated) in the Afferdensche en Deestsche Waarden (SSEO). 6h Tenax-extractable (fast-desorbing) concentrations and lake characteristics (including BC in sediment) were also determined Results were modelled using a BC-inclusive steady state bioaccumulation model (Moermond et al. 2005).
2. *Modelling the effect of BC on accumulation of POPs in food webs representing floodplain lakes, in relation to metabolic transformation.*
   Two main issues in HOC bioaccumulation modelling were evaluated: (1) limited availability due to strong sorption to BC; and (2) the role of metabolic transformation. Data for the model validation was taken from an SSEO-model ecosystem experiment with historically contaminated sediment. First, a food web accumulation model was constructed for macrophytes, periphyton, flab (floating algal biomass), zooplankton, invertebrates and fish. The original model (Traas et al. 2004) included a matrix food web structure. It was extended with sorption to BC (see also Chapter 14 for the model definition and some examples of its usefulness). This extended model was validated for PCBs in all biota and for those PAHs that are not metabolized. After this validation, the same model parameters were used to optimize the model for the metabolic transformation rate of PAHs (Moermond et al. in prep.; Traas et al. 2004).
3. *Modelling the effect of BC on accumulation in freshwater, estuarine and marine food chains, including probabilistic modelling of uncertainty.*

Due to strong sorption to black carbon, dissolved concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) in water are lower than predicted by traditional equilibrium partitioning. This deviation also influences bioaccumulation modelling and risk assessment. We quantified the reduction in Biota-Solids Accumulation Factors (BSAFs) of PAHs due to sorption to black carbon by extending the mechanistic bioaccumulation model OMEGA (Hendriks et al. 2001). Uncertainties with respect to variation in the BC related model parameters were analyzed using Monte Carlo simulations (Hauck et al. in prep.).

10.3 Main results

1. Modelling the effect of BC on steady state BSAFs for POPs in macro-invertebrates.
BSAFs could be explained with a model including a term for Freundlich sorption to BC and a term for uptake from fast-desorbing concentrations in ingested sediments (Figure 14). Freundlich coefficients for in situ sorption to BC (K_F) were calculated from slow-desorbing fractions and BC contents and agreed well with literature values for K_F. Furthermore, in contrast to BSAFs based on total extracted concentrations, Tenax-based BSAF showed a strong positive correlation with log K_{OW}. We therefore argue that BC caused slow desorption and limited BSAFs in these lakes. Seasonal and lake effects on BSAFs were detected, while the differences between oligochaetes and other invertebrates were small for PCBs and within a factor of 10 for PAHs. BSAFs for pyrogenic PAHs were much lower than for PCBs, which was explained by stronger sorption to BC, lesser uptake from ingested sediment, and metabolic transformation.

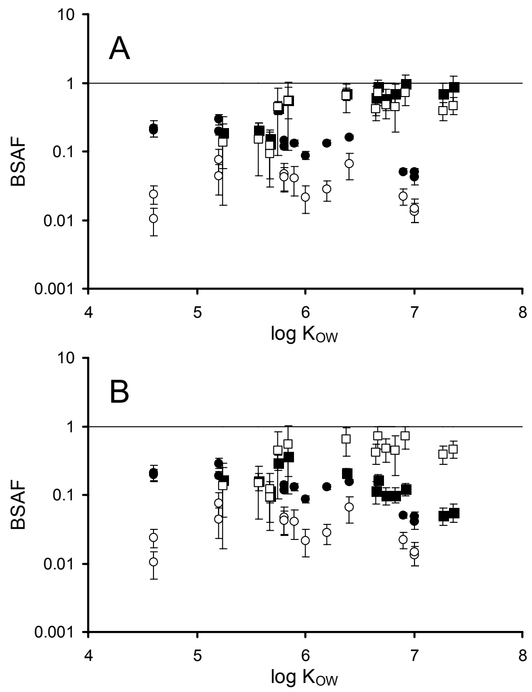


Figure 14. Measured and modelled BSAF values for PAHs (○/●) and PCBs (□/■). Measured values have open markers. Model results are shown with a food ingestion term (panel A) versus no-food ingestion term (panel B). Results for all lakes and seasons (n = 11) are averaged and standard errors are shown. The solid line represents the conventional BSAF value of 1.

2. *Modelling the effect of BC on accumulation of POPs in food webs representing floodplain lakes, in relation to metabolic transformation.*
The results show that without a term for sorption to BC, the BSAF data for PAHs are greatly overestimated (Figure 15 A and B, upper curves). Furthermore, it appears that including sorption to BC brings the model curve to the correct order of magnitude (Figure 15 A and B, middle curves), whereas for the more hydrophobic PAHs, metabolization should also be included (Figure 15 A and B, lower curves). Metabolic transformation rates for PAHs were optimized to be on average 2.51 day-1 for fish and 0.193 day-1 for invertebrates, which is consistent with literature reports.

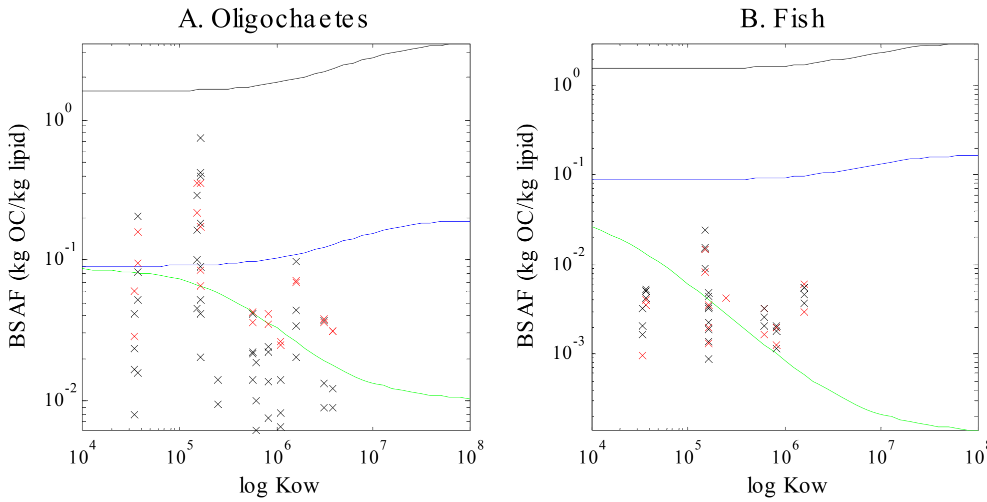


Figure 15. PAH bioaccumulation in oligochaetes (Panel A) and fish (Panel B) as a function of K_{OW}: comparison of model simulations (1, upper curve) excluding sorption to BC and excluding metabolic transformation; (2, middle curve) including sorption to BC; and (3, lower curve) including sorption to BC and including metabolization. Markers indicate individual measured BSAFs in n = 6 freshwater model ecosystems representing floodplain lakes. For fish, less data are available, because at higher log K_{OW}"s, values were below detection limits.

3. *Modelling the effect of BC on accumulation in freshwater, estuarine and marine food webs, including probabilistic modelling of uncertainty.*
By including BC in the OMEGA model, BSAFs typically decreased by two orders of magnitude and were more in line with field data in marine, fresh water and terrestrial ecosystems. Probabilistic modelling showed that uncertainty in BSAFs, when sorption to black carbon is included, is about three orders of magnitude. This uncertainty is mainly caused by unknown black carbon contents in the sediment (which played no role in the aforementioned studies 1 and 2). This implies that including measurements of black carbon contents is relevant in reducing uncertainty in estimating BSAFs of PAHs.

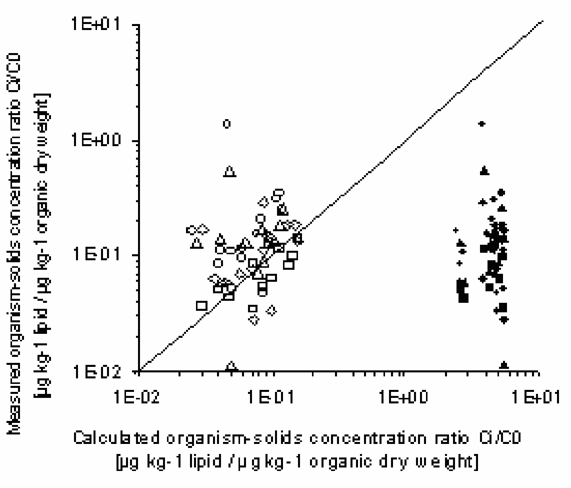


Figure 16. Measured BSAFs for PAHs versus OMEGA estimations for the concentration in solids, organic matter fractions and black carbon fractions measured by Hauck et al. (2007) and Van den Heuvel-Greve et al. (2006). The straight line gives the hypothetical 1:1 match of measured and estimated data:
♦ for *Arenicola marina* to sediment concentration ratios without black carbon correction;
◊ for *Arenicola marina* to sediment concentration ratios with black carbon correction;
■ for *Cerastoderma edule* to sediment concentration ratios without black carbon correction;
□ for *Cerastoderma edule* to sediment concentration ratios with black carbon correction;
● for *Arenicola marina* to suspended solids concentration ratios without black carbon correction;
○ for *Arenicola marina* to suspended solids concentration ratios with black carbon correction;
▲ for *Cerastoderma edule* to suspended solids concentration ratios without black carbon correction;
Δ for *Cerastoderma edule* to suspended solids concentration ratios with black carbon correction.

10.4 Implications and possible use

The current results together with earlier work (Koelmans et al. 2006) provide a robust body of evidence for the thesis that bioaccumulation of POPs in sediments is partly driven by the presence of BC, and that it usually is much lower than predicted by the Equilibrium Partitioning Theory approach. The effect is strongest at sites with relatively high BC levels and for planar POPs in the nanogram and picogram per litre aqueous concentration range. As these are the environmentally relevant sites and concentrations, and planar POPs usually are the most toxic chemicals, this has important implications for the assessment of ecological risks and remediation strategies for contaminated sediments. First, the presence of BC lowers exposure and toxicological risks of POPs. This means that ecological target values will be reached much earlier. Second, the presence of BC may have a positive implication for sediment remediation. Remediation strategies aim at lowering total sediment concentrations until the standards, which are set by the authorities, are reached. BC may lead to stronger fixation to the sediment, which implies that the current standards probably are too safe. Hence, standards could be re-evaluated and may safely be set to site-specific higher POP levels. Alternatively, standard systems could be reset to account for BC sorption, for example by basing standards on freely dissolved aqueous concentrations rather than total concentrations in the sediment. It is recommended that these perspectives are explored further in future national and international research programmes. The most important rationale is that cost savings may be in reach, since the need for sediment remediation may be less.

11 Metal accumulation in the earthworm *Lumbricus rubellus*: model predictions compared to field data

By: K. Veltman , M. Huijbregts, M. G. Vijver, W.J.G.M. Peijnenburg, P.H.F. Hobbelen, J.E. Koolhaas, C.A.M. van Gestel, P. van Vliet & A. J. Hendriks

11.1 Problem definition

Environmental risk assessment procedures, including those for the derivation of quality criteria in the Netherlands, often include bioaccumulation of metals by using a single value for the bioconcentration factor (organism-soil concentration ratio), which is invariant to differences in soil characteristics, soil concentrations and species variability. As a result, risks may be under or overestimated by more than an order of magnitude. Incorporation of bioaccumulation models that account for these differences in a simple and transparent way would improve risk assessment procedures by allowing management to select the correct priorities (sites, chemicals) without spending budget on remediation of soils and sediment that pose a small risk. Metal accumulation in biota is influenced by several interacting factors. Environmental conditions, such as pH and organic matter content, determine the bioavailability of metals in soil, whereas metal- and species-specific characteristics influence uptake and elimination rates. As a result, field accumulation studies have provided widely different information depending on the ecosystems, species and conditions investigated. In this study, we improved and tested the bioaccumulation model OMEGA (Optimal Modelling for Ecotoxicological Applications – consisting of internationally accepted relationships) on earthworms in floodplain soil. The metals studied are the essential metals zinc (Zn) and copper (Cu), and the non-essential metals cadmium (Cd) and lead (Pb). The study is described in detail by Veltman et al. (2007b) .

11.2 Approach

Internal metal concentrations predicted by OMEGA were compared to field accumulation data from various locations in the Netherlands. Earthworms predominantly accumulate metals via pore-water mediated dermal uptake. Ingestion of soil is therefore excluded as a route of uptake for earthworms; steady-state internal concentrations were calculated as the influx via water, divided by the elimination rate. Earthworms rely on compartmentation as a main detoxification mechanism for metals. This influences elimination kinetics, as strongly bound substances may hardly be eliminated via “normal” elimination pathways such as excretion and egestion. Therefore, two elimination rate constants were calculated for each metal. Firstly, a maximum elimination rate, representing elimination via excretion, egestion and growth dilution, was predicted. Secondly, a minimum elimination rate was obtained by assuming that metals are only eliminated via growth dilution. Linear regression analysis was performed to relate metal concentrations in earthworms to total soil concentrations. The slope and intercept of these regressions were compared to model lines.

11.3 Main results

Our validation to field accumulation data (Figure 17) shows that OMEGA accurately predicts internal cadmium concentrations. However, incorporation of regulation in the model is necessary to improve predictability of the essential metals zinc and copper.

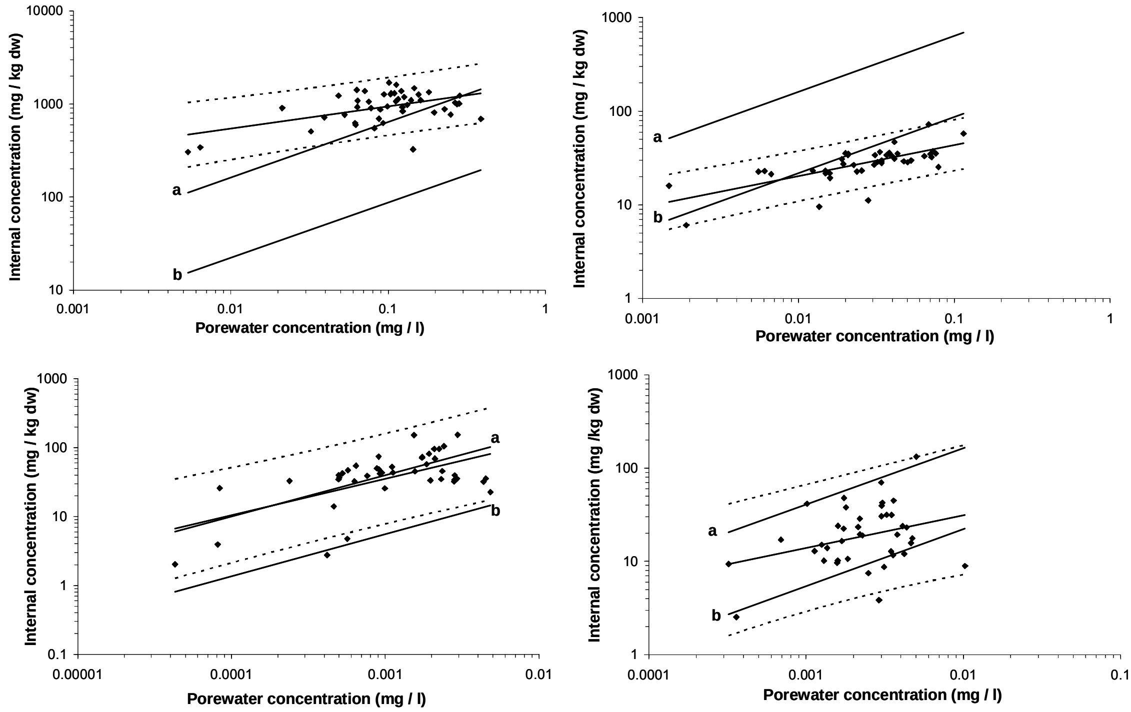


Figure 17. Measured internal metal concentrations in *Lumbricus rubellus* (C_i, mg·kg^{-1} dry body weight) plotted against estimated pore water concentrations (C_{pw}, mg·L^{-1}), compared to OMEGA model predictions. The solid line represents regression for field data. Solid lines a and b represent, respectively, (a) minimum elimination (growth dilution only) and (b) maximum elimination (sum of egestion, excretion and growth dilution). The dashed lines represent the 97.5th and 2.5th percentiles of the field data. Top left: zinc. Top right: copper. Bottom left: cadmium. Bottom right: lead.

11.4 Implications and possible use

The results show that internal metal concentrations in the earthworm have less than a linear (slope < 1) relationship to the total concentration in soil, while risk assessment procedures often assume the biota-soil accumulation factor (BSAF) to be constant.

In environmental risk assessment procedures OMEGA can be used to predict cadmium accumulation in earthworms, either as a whole or by incorporating the validated equations into other models and procedures.
Additionally, the model can be used to facilitate interpretation and underpin the correctness of field data on cadmium accumulation. At present, application for essential metals is limited, as regulation of uptake and/or elimination rate constants is not yet incorporated in the model.

Finally, the study is a fine example of how data obtained by different research groups, using different methods, can efficiently be combined and structured in a simple model, to facilitate use in environmental management.

12 Modelling cadmium accumulation in a herbivorous and carnivorous terrestrial food chain

By: K. Veltman, M. Huijbregts, T. Hamers, S. Wijnhoven & A.J. Hendriks

12.1 Problem definition

Environmental risk assessment procedures, including those for quality standards in the Netherlands, often include bioaccumulation of metals using a single value for the biomagnification factor (organism-food concentration ratio), invariant to differences in food characteristics, food concentrations and species variability. As a result, risks may be substantially under or overestimated. Incorporation of bioaccumulation models that account for these differences in a simple and transparent way improves risk assessment procedures, allowing management to select the correct priorities (sites, chemicals) without spending budget on remediation of soils and sediment that pose a small risk.

Metal accumulation in biota is influenced by several interacting factors. Environmental conditions, such as pH and organic matter content, determine bioavailability of metals in soil, whereas metal- and species-specific characteristics influence possibilities for food chain transfer. As a result, field accumulation and biomagnification studies have provided widely different information depending on the ecosystems, species and conditions investigated. In this study, we have improved and tested the bioaccumulation model OMEGA (Optimal Modelling for Ecotoxicological Applications – consisting of internationally accepted relationships) on field accumulation data of cadmium in the carnivorous shrew *Sorex araneus* and the herbivorous voles *Clethrionomys glareolus* and *Microtus agrestis*. Additionally, we compared predicted uptake and elimination rate constants to empirical values. The study is described in detail by Veltman et al. (2007a).

12.2 Approach

Field accumulation data were collected for the carnivorous common shrew (*Sorex araneus*) and two herbivorous voles, *Microtus agrestis* and *Clethrionomys glareolus*. These field data comprised several studies and locations, mainly diffusively polluted floodplain soils and former mining areas. Studies were selected if cadmium concentrations in food items, i.e. earthworms and plants, were measured. because small mammals predominantly accumulate cadmium via ingestion, absorption of water and inhalation of air were excluded in the modelling approach. Binding of cadmium to the metal-binding protein metallothionein is the main detoxification mechanism of small mammals and results in very low elimination rates. Therefore, a minimum elimination rate was estimated, since as elimination via growth dilution only. Linear regression analysis was performed to relate cadmium concentrations in the kidneys, livers and whole bodies of small mammals to total soil concentrations.

12.3 Main results

The results show a significant relationship between total soil concentrations and Cd concentrations in kidneys and livers of carnivorous shrew and herbivorous voles (Figure 18, only kidney data shown). Cadmium concentrations in above-ground parts of plants are generally lower than concentrations in

Carnivorous shrew
Herbivorous voles

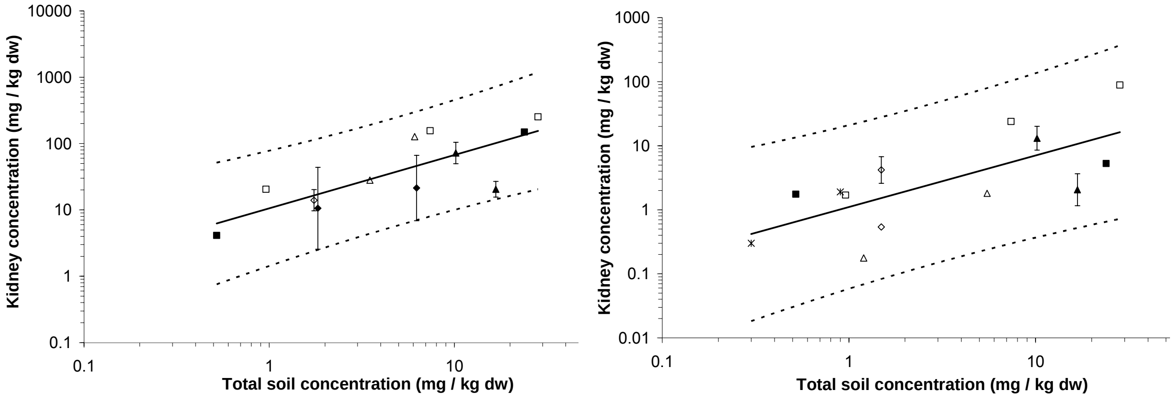
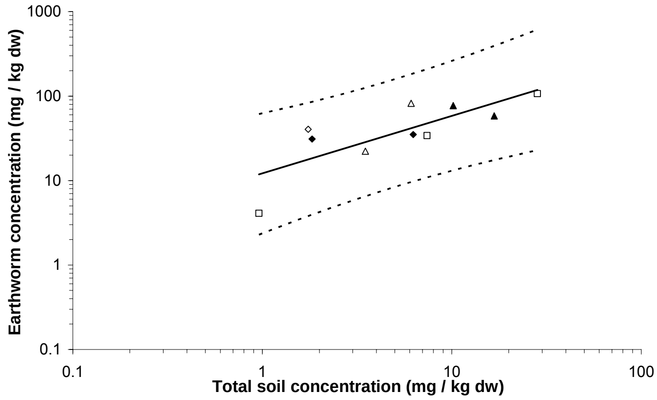


Figure 18. Cd accumulation in kidneys of carnivorous shrew (a) and herbivorous voles (b) (mg×kg^{-1} dw) compared to total soil concentrations (mg×kg^{-1} dw). ∫ Biesbosch (Hamers et al. 2006), ◆ Rhine (Hendriks et al. 1995), ⎨ ADW (Van Vliet et al. 2005; Wijnhoven et al. 2007; Wijnhoven et al. 2006a), ⎧ near closed smelter (Budel) and industrially polluted area (Arnhem) (Ma et al. 1991), ƒ near Cd / Cu refinery, 1 km from refinery and reference location (Hunter et al. 1987a; Hunter et al. 1989), ■ near mine and reference location in UK (Andrews et al. 1984; Shore 1995), * lead mine (Frongoch) and reference site (Milton et al. 2003). Solid line represents empirical regression. Upper and lower dashed lines represent the 97.5th and 2.5th percentile of the field data, respectively. The error bars represent the 95% confidence intervals and were plotted when possible.

a. Cadmium concentrations in earthworms
b. Cadmium concentrations in various plant species* (next page)



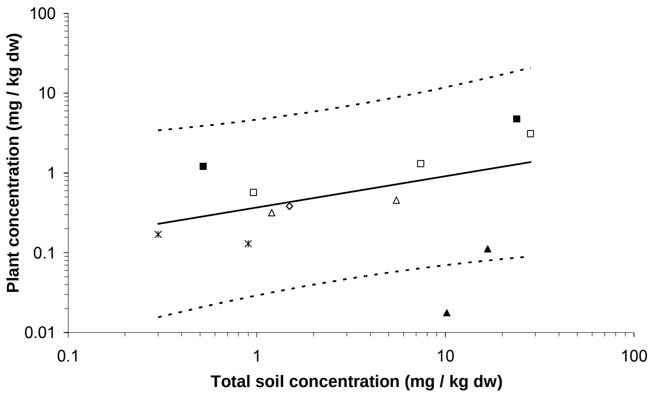


Figure 19. Empirical cadmium concentrations in (a) earthworms (mg.kg^{-1} dw) and (b) plants (mg.kg^{-1} dw) versus total soil concentrations (mg.kg^{-1} dw). ∫ Biesbosch (Hamers et al. 2006), ◆ Rhine (Hendriks et al. 1995), { ADW (Van Vliet et al. 2005; Wijnhoven et al. 2007; Wijnhoven et al. 2006a), ʃ near closed smelter (Budel) and industrially polluted area (Arnhem) (Ma et al. 1991), near Cd / Cu refinery, 1 km from refinery and reference location (Hunter et al. 1987a; Hunter et al. 1987b), ■ near mine and reference location in UK (Andrews et al. 1984; Shore 1995), * lead mine (Frongoch) and reference site (Milton et al. 2003). Solid line represents empirical regression. Upper and lower dashed lines represent the 97.5th and 2.5th percentile of the regression model, respectively.

earthworms at equal soil levels (Figure 19). This is in agreement with the observation that Cd concentrations in voles are generally lower than in the shrew. Additionally, a large variation in cadmium levels in plants is observed when related to total soil concentrations. As a consequence, regressions of Cd accumulation in herbivorous voles have a lower explained variance compared to carnivorous shrews based on total soil concentrations.

Preliminary model results show that the elimination rate constant is accurately predicted by OMEGA (Table 4).

Table 4. Predicted elimination rates (kx,ex in d^{-1}) compared to empirical rate constants

	Empirical $k_{x,ex}$ in d^{-1}	OMEGA $k_{x,ex}$ in d^{-1}	Reference
Sorex araneus		$6.8 \cdot 10^{-3}$	
Microtus agrestis		$5.1 \cdot 10^{-3}$	
Mus musculus	$9.4 \cdot 10^{-3}$		Jörgensen (1979)
Rattus norvegicus	$2.3 \cdot 10^{-3}$		Jörgensen (1979)
"rodents"	$3.5 \cdot 10^{-3} – 9.0 \cdot 10^{-4}$		Friberg et al. (1986)
"mice"	$2.9 \cdot 10^{-3}$		Engström and Nordberg (1979)

12.4 Implications and possible use

Total soil concentrations are a good predictor for cadmium accumulation in the carnivorous shrew. A lower predictability is observed for herbivorous voles, which is mainly due to the considerable variation in cadmium accumulation in above-ground parts of plants. In environmental risk assessment procedures, OMEGA can be used to predict cadmium accumulation in earthworms, either as a whole or by incorporating the validated equations into other models and procedures. The mechanistic modelling and calibration of uptake rate constants is currently being carried out. An advantage of using the model instead of empirical regressions is that the model allows extrapolation to other species and possibly other metals, whereas regressions are species- and metal-specific.

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13 Population growth and development of the earthworm *Lumbricus rubellus* in a polluted field soil, and possible consequences for the godwit

By: C. Klok

13.1 Problem definition

Many soils are polluted with mixtures of moderate levels of contaminants. In the Netherlands, 175,000 sites in rural areas are classified as highly polluted. However, it remains unclear to what extent local ecosystems are endangered. In this paper, we report on the effect of contaminants on earthworms in a meadow system and the possible consequences for the godwit. We tested field soil from a site polluted with a mixture of contaminants and soil from a reference site with similar soil characteristics and agricultural use. In the polluted soil, copper, mercury, and lead were elevated by more than 200% compared with the reference soil. The study is described in detail by Klok et al. (2006a).

13.2 Approach

Bioassays on growth and reproduction in the earthworm species *Lumbricus rubellus* were conducted in both soils, and a population model (PODYRAS) was used to assess the population-level consequences of changes in growth and reproduction.

13.3 Main results

In the reference soil, pollutant levels were below target values. No significant effects were seen on reproduction and survival in *L. rubellus*, but development was retarded in the polluted soil (Figure 20). This resulted in a 23% lower growth rate and a change in demography toward younger individuals.

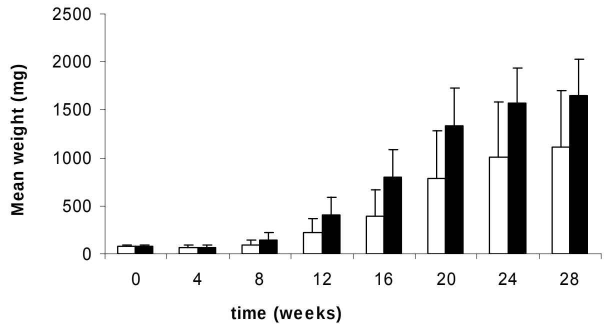

Figure 20. Growth of *Lumbricus rubellus* hatchlings in two Dutch field soils during eight months, mean individual weights and standard deviation. Black bars: reference soil (Zeevang); white bars: polluted soil (Blokland).

13.4 Implications and possible use

The effects on the earthworm population may have consequences for the godwit (*Limosa limosa*), which mainly feeds on earthworms during the breeding season. Field data on population composition of earthworms were used to support the laboratory results. As shown in this paper, individual growth can influence reproductive output. If individual growth is retarded, maturation is delayed. Treatment of growth and reproduction as independent endpoints could therefore lead to an overestimation of the population growth rate. The Dynamic Energy Budget (DEB) theory, developed by Kooijman, offers a solid mechanistic general theory that integrates different individual-level endpoints. This theory is valuable for ecotoxicological assessments.

14 Food web modelling of toxicant exposure and addressing functional groups

By: T. P. Traas & T. Aldenberg

14.1 Problem definition

This study concerns a model to predict the ecosystem response to chronic pollutant stress. The method incorporated results from single species tests in a food web model based on functional groups. Although the protection criteria for ecosystems are usually defined at the level of populations and ecosystems, current methods give limited information on ecological interactions and habitat factors, or on the specific importance of keystone species and functional groups. Tests in experimental ecosystems or microcosms showed that ecological redundancy can mask toxic effects on ecosystem function, but that the species composition changes and sensitive species disappear.

To predict these findings, and to better understand them, a terrestrial food web model, SimpleChain, was developed. This model describes the energy flow in a tri-trophic food web (Klepper et al. 1999). It was adapted to incorporate the different sensitivity of functional groups, described by Species Sensitivity Distributions (SSDs) for specific chemicals (see Chapters 15 and 29 for details on the SSD model). The data for this study were obtained from a previous semi-controlled field study outside the SSEO programme (Korthals et al. 1996), in which soil animals were exposed to copper for a long period.

14.2 Approach

The food chain model describes the long-term effects of persistent chemicals in terrestrial food chains. It focuses on three aspects:

- the interaction between toxicant stress,
- competition between species, and
- the role of biodiversity.

The model allows predictions on species replacement and ecosystem function for different chemicals, using available toxicity data for different species groups. The model takes into account that species compete for resources in the ecosystem and that chemicals influence the competitive ability of species. Effects of chemicals on individual species thus disturb the "normal" pattern of competition, leading to a replacement of affected species by insensitive species. With increasing toxicant concentrations, insensitive species start to dominate, and the efficiency of the system to process the natural resources decreases.

The effect of the toxicant on the ecosystem was studied by running the model with a range of concentrations. The toxicant randomly affects species within a functional group, because the sensitivity is randomly drawn from the SSD. If the species happens to be the most efficient, it was the winner in competition with less-efficient species. However, due to the toxicant effects, it will now become less efficient. The model calculates which of the species within the functional group then becomes the most efficient; this is probably one that is less sensitive to the toxicant and thus not affected by it. Due to the toxicant, the sensitive species is thus effectively out-competed by another, less sensitive one (Figure 21). This process is repeated for each parallel food chain in the model, for each concentration in the concentration range that we studied. By varying the number of species within a functional group, we could see how this affected the stability of the ecosystem in relation to toxicant stress.

14.3 Main results

After it was developed using the data from the aforementioned study, the model was run using cadmium as an example. The group of detritus eaters consisted of 5 species in this example, with sensitivities randomly drawn from the SSD for cadmium sensitivity of invertebrates (left line in Figure 21A). These detritus eaters are represented by several species (Figure 21B). At higher concentrations, the insensitive species remain, which eventually are reduced to the last surviving species (no. 2 in Figure 21). It is interesting to see that the relationship between biomass and species diversity is erratic; even though only a few species survive at higher cadmium concentrations, the biomass itself is not necessarily reduced. However, it should be noted that this pattern looks different for each new probabilistic simulation and is not a general pattern.

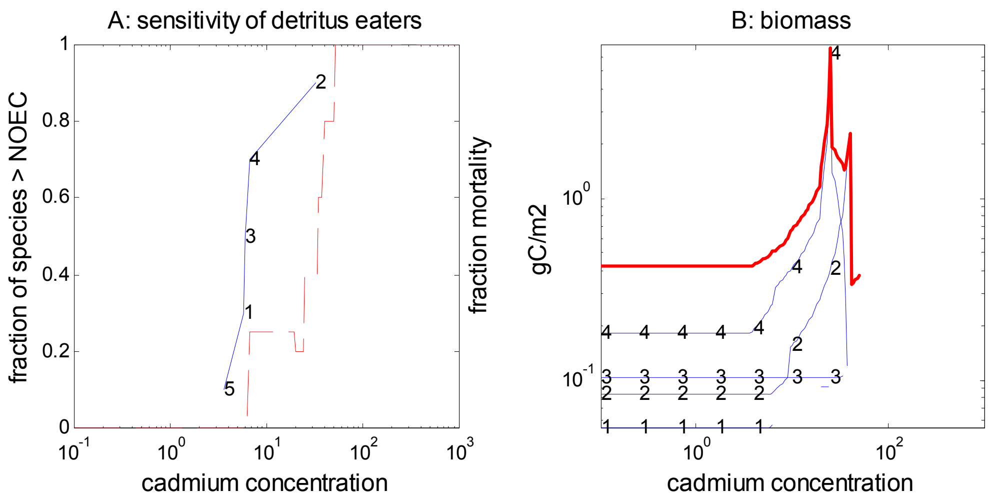

Figure 21. Cd sensitivity for 5 detritus eaters, randomly drawn from the detritus eater SSD (panel A). With increasing concentrations, sensitive species are replaced (numbered lines in panel B), while the overall biomass (fat, top line) is only affected at high concentrations.

The SSD that was derived from single-species test results is shown as the smooth line on the left of Figure 22A-B. The model predicts that species can be affected very quickly after their No Observed Effect Concentration (NOEC) has been exceeded. Due to the competition with other species sharing the same energy resources, the species will go extinct, sometimes at concentrations close to the NOEC. The probability that this happens is indicated by the dotted lines surrounding the average mortality probability in Figure 22. The figure indicates that sensitive species can go extinct even if their NOEC is only mildly exceeded. If a species gets weaker due to the toxic chemical, other species can take advantage. Even if the toxicant does not kill immediately, the combination with increased competition pressure will[13]. The model thus allows for an estimation of the mortality of species, taking ecosystem interactions into account.

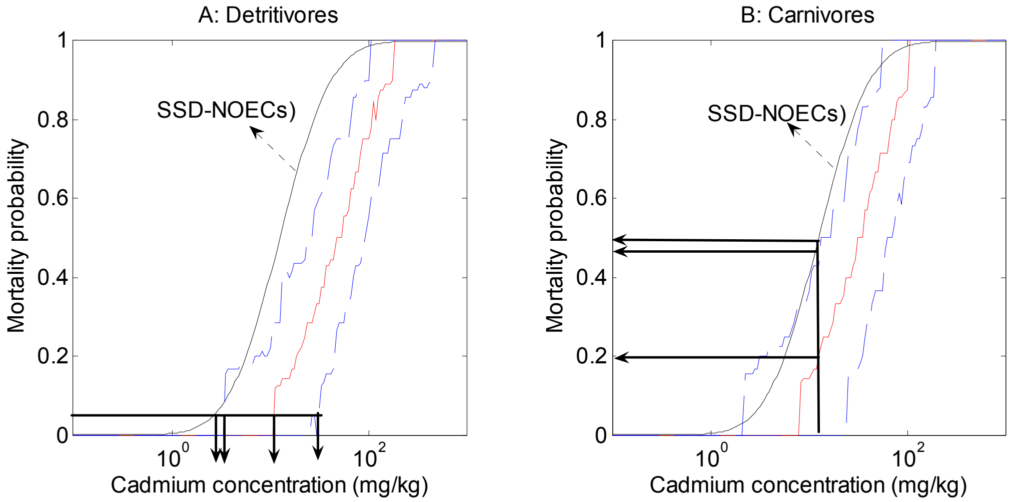

Figure 22. Effect of cadmium on the mortality of detritivores and carnivores in a terrestrial food web model in comparison to SSD-NOECs (smooth lines). The "broken" middle curves represent the average mortality probability, the "broken and dotted" outer lines indicate the confidence interval surrounding the estimate. The confidence interval for a given mortality probability (e.g., 5% in panel A) can be read on the concentration (x) axis. The mortality probability at a given concentration can be read on the probability (y) axis (panel B).

14.4 Implications and possible use

The model predicts that biodiversity can be affected very quickly above the NOEC of sensitive species. This leads to a loss of sensitive species, although ecosystem function is not directly affected. Even with fewer species, the biomass within a functional group is not necessarily reduced. This simply means that the ecosystem still functions more or less properly under moderate toxicological stress, but fewer species are present. This makes the system more sensitive and vulnerable due to additional stress factors (disturbance, temperature etc.).

The model can be used to estimate the impact of background concentrations in agro-ecosystems or natural systems, based on available toxicological data for different functional groups in the model (e.g. from ecotoxicity databases). In this way, the probability of species extinction due to toxic stress can be estimated as a first indication for the concentration range where this could be observed in the field. Although the model is an idealized blueprint of a terrestrial ecosystem, field data showed good agreement with model predictions.

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[13] When SSEO studies resulted in a low magnitude of adverse effects, this may in part be explained by this phenomenon: the most sensitive species have disappeared, and the local gradient does not induce further species loss.

15 Predictive value of Species Sensitivity Distributions for effects of pesticides in freshwater ecosystems

By: P.J. Van den Brink PJ, L. Maltby, N. Blake & T.C.M. Brock

15.1 Problem definition

The challenge faced by many ecological risk assessors is to derive threshold concentrations for environmental contaminants in order to protect species diversity and the functional attributes of natural ecosystems. Species vary markedly in their sensitivity to environmental contaminants, and this variation can be described by constructing a species sensitivity distribution (SSD). This is a statistical distribution, estimated from a sample of toxicity data and visualized as a cumulative distribution function. Species Sensitivity Distributions are used to calculate the concentration at which a specified proportion of species will be affected, referred to as the HCp, the Hazardous Concentration for p% of species. The most frequently estimated hazardous concentrations are the HC5 and HC10; the HC5 derived from an SSD of No Observed Effect Concentration (NOEC) values being used to define the Maximum Permissible Concentration (MPC) for environmental contaminants in the Netherlands.
One of the assumptions of the SSD approach that has been criticized is that laboratory conditions do not greatly influence the sensitivity of species, and that the hazardous concentration derived from the SSD will therefore be sufficient to protect ecosystems. Sensitivity distributions are constructed using data from single-species laboratory toxicity tests, but the derived hazardous concentration is applied to multi-species assemblages in natural ecosystems. By definition, single-species tests exclude interactions between species. Their use in risk assessment consequently assumes that interactions among species in communities can also be ignored or accounted for. The aim of this study was therefore to assess how SSDs derived from single-species laboratory toxicity data could be used to protect species assemblages in aquatic ecosystems. This was achieved by comparing SSDs constructed from “natural” and artificial assemblages exposed in micro- and mesocosms and single-species laboratory tests, respectively. The hazardous concentrations derived from single-species SSDs were then compared to threshold levels for the direct toxic effects of pesticides in freshwater micro- and mesocosms. The study is described in detail by Maltby et al. (2005).

15.2 Approach

The aim of the study was addressed using aquatic toxicity data for 16 insecticides and 9 herbicides, including organophosphate, pyrethroid, organochlorine, carbamate benzylphenyl urea compounds and triazines. Sensitivity distributions were constructed using acute toxicity data and described by a log-normal model. Previous studies have used chronic toxicity data to construct SSDs. However, acute toxicity data have a number of advantages over chronic toxicity data. First, for most chemicals there are insufficient chronic toxicity data to generate appropriate SSDs, but there may be sufficient acute toxicity data. Second, whereas acute toxicity data relate to a limited number of responses and time scales (e.g. 96-h median lethal concentrations), chronic toxicity data include a wide range of responses and test durations, thereby introducing additional variability into the SSD. Third, pesticide risks are often short-term and therefore more appropriately assessed using acute toxicity data.

A log-normal model was fitted to a minimum of six data points, and the resulting distribution was used to estimate lower (95% confidence), median (50% confidence), and upper (5% confidence) 5% HC (HC5) values. These HC5 values were compared to the response of pesticide-stressed freshwater ecosystems using a similar exposure regime. Data on the toxicity of selected pesticides under field and semi-field conditions was taken from Brock et al. (2000a; Brock et al. 2000b) and updated using information from open literature and industry. The papers of Brock et al. contain a review of all experiments performed in microcosms and mesocosms published between 1979 and 2000 that evaluated the effects of pesticides. For each concentration tested, Brock et al. classified the effects on seven structural endpoints (i.e. macrophytes, periphyton, phytoplankton, zooplankton, molluscs, macrocrustaceans and insects combined, and fish and tadpoles combined) and one functional endpoint (community metabolism). Effects were assigned to five classes: Class 1, no effect; Class 2, slight effect usually on a single sampling date immediately after application only; Class 3, clear short term effect (recovery within 8 weeks post last application); Class 4, clear effect duration unknown; Class 5, clear long lasting effects (no recovery within 8 weeks post last application). Because recovery is a process that can never be accounted for by using the SSD concept, for the our paper we combined Classes 3 to 5 into one new effect class 3, named “clear effects”.

15.3 Main results

The taxonomic composition of the species assemblage used to construct the SSD does have a significant influence on the assessment of risk. In case of insecticides, hazardous concentrations estimated using laboratory-derived acute toxicity data for freshwater arthropods (the most sensitive taxonomic group to insecticides) were compared to the response of freshwater ecosystems exposed to insecticides. The sensitivity distributions of freshwater arthropods were similar for both field and laboratory exposure, and the lower HC5 (95% protection with 95% confidence) estimate was protective of adverse ecological effects in freshwater ecosystems. The corresponding median HC5 (95% protection level with 50% confidence) was generally protective of single applications of insecticide, but not of continuous or multiple applications. In the latter cases, a safety factor of at least five should be applied to the median HC5. In case of herbicides, primary producers proved to be the most sensitive group, therefore only sensitive primary producers should be included in the risk assessment of herbicides. The lower limit of the acute HC5 and the median value of the chronic HC5 were protective of adverse effects in aquatic micro/mesocosms even under a long-term exposure regime. The median HC5 estimate based on acute data was protective of adverse ecological effects in freshwater ecosystems when a pulsed or short-term exposure regime was used in the microcosm and mesocosm experiments (Figure 23).

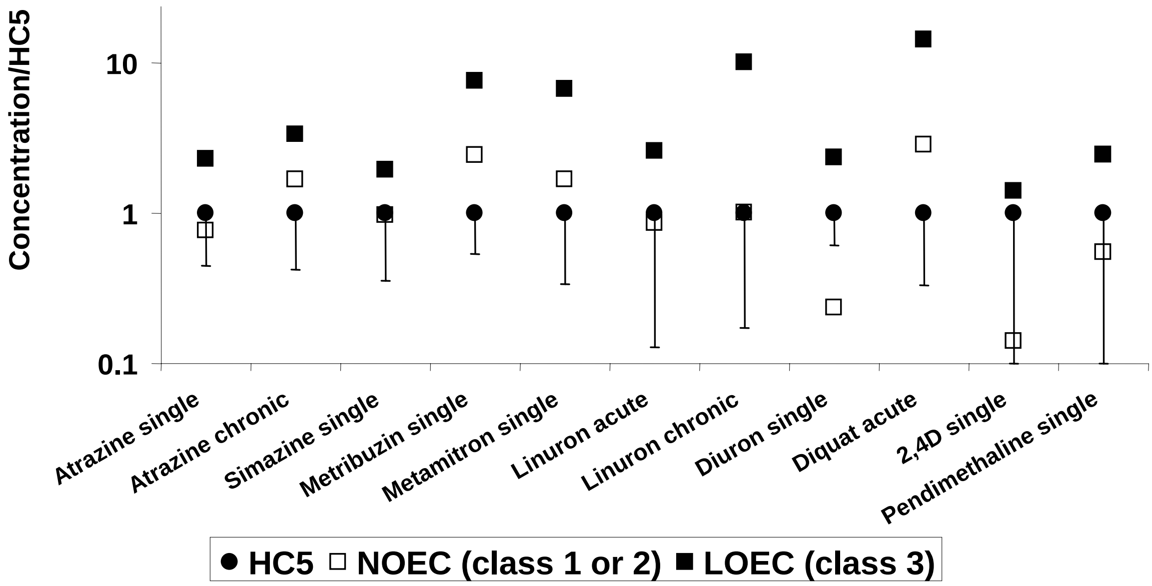

Figure 23. Comparison of single-species HC5 estimates (hazardous concentration for 5% of species) and response of multispecies assemblages in micro- and mesocosms exposed to herbicides. Median HC5 estimates (solid circles) and lower (95% confidence) estimate (line) were calculated using freshwater primary producers short-term toxicity data. Filled squares represent the LOECeco (ecosystem lowest observed effect concentration, Class 2), open squares the NOECeco (ecosystem no observed effect concentration, Class 1) concentration derived from micro- and mesocosm studies. Note that for all comparisons, both the compound and the exposure regime used in the micro- and mesocosm experiment are noted. For clarity, the NOECeco and LOECeco are standardized on the HC5.

15.4 Implications and use

The hesitation of risk assessors to use models to estimate the effects of pesticides explains why a relatively simple concept such as the SSD is disputed in the arena of ecological risk assessment (see Posthuma et al. (2002b) for an extensive overview). This is not without reason, as the results of an SSD largely depend on the way the toxicity data are processed. In these papers we therefore made an empirical comparison between expected sensitive and non-sensitive species, standard and non-standard test species, acute and chronic toxicity and the laboratory and field toxicity of pesticides. In all these comparisons, SSD was used to describe sensitivity at the community level. This work proved that the HC5 can be used in the risk assessment of pesticides, if recovery is not of concern. The latter is an important qualification, since small effects may be acceptable if recovery is completed within a certain time frame. The aspect of recovery, however, can of course not be addressed by SSD.

16 Impact of TriPhenylTin-Acetate (TPT-Ac) in microcosms simulating floodplain lakes: comparison of species sensitivity distributions between lab and microcosm

By: I. Roessink, J. D. M. Belgers, S. J. H. Crum, P. J. van den Brink & T. C. M. Brock

16.1 Problem definition

Relatively few published studies have dealt with the ecological risks of fungicides to freshwater communities (Cuppen et al. 2000). Although several studies on the fate and effects of fungicides in aquatic ecosystems have recently been published, knowledge of the ecological impact of fungicides is still limited. Several of the fungicides studied to date appear to have biocidal properties, and the fact that some of these compounds may also exhibit endocrine-disrupting abilities has certainly drawn attention to this group. An example of this group can be found in the organotins, which are amongst the more frequently studied biocides. The present study deals with the organotin compound triphenyltin acetate (TPT), a fungicide for which little adequate freshwater laboratory toxicity data, and no field or semi-field toxicity data, had been published.

The study objectives were to shed light on the types of freshwater organism that are sensitive to triphenyltin acetate (TPT) and to compare the laboratory and field sensitivities of the invertebrate community. To attain these objectives, SSDs based on laboratory toxicity data and on data obtained from a semi-field experiment were compared. The study is described in detail by Roessink et al. (2006a).

16.2 Approach

The responses of a wide array of freshwater taxa (including invertebrates, phytoplankton and macrophytes) were studied in acute laboratory single species tests. The results of these tests (in the form of EC50s) were compared with the concentration–response relationships of aquatic populations observed in two types of freshwater microcosms.

An outdoor microcosms experiment with TPT was performed using a total of 20 concrete cosms (length 140 cm, width 120 cm, and height 80 cm) with a water column of approximately 50 cm and a sediment layer of approximately 10 cm (Roessink et al. 2006b). The microcosm experiment aimed to compare the ecological impact of a single application of TPT between test systems with clean and systems with polluted sediments derived from river floodplain lakes. The polluted sediment contained higher levels of nutrients, metals, PAHs, PCBs, and organic carbon. The experiment used a regression design with five duplicate single applications of TPT (controls, 1, 10, 30 and 100 µg/l) per sediment type. Responses of populations of macroinvertebrates, zooplankton, phytoplankton and macrophytes were studied at several time intervals after TPT application. Since no major differences in community responses between systems were observed (Roessink et al. 2006b), only the clean sediment systems were used for the comparison with the response in the laboratory. For a detailed description of the design and results of the microcosm experiment, see Roessink et al. (2006b). Field EC50s could be calculated from the responses observed in the microcosms.

In this way, SSDs based on laboratory data could be compared to a field-derived SSD, which allowed the comparison of laboratory and field toxicity of TPT.

16.3 Main results

Representatives of several taxonomic groups of invertebrates, and several phytoplankton and vascular plant species proved to be sensitive to TPT, illustrating its diverse modes of toxic action. Statistically calculated ecological risk thresholds (HC5 values) based on 96 h laboratory EC50 values for invertebrates were 1.3 (90% C.I. 0.4–3.0) ng/l. Based on microcosm species sensitivity distributions (SSD) for invertebrates in sampling weeks 2 after TPT treatment, the value was 0.4 (90% C.I. 0.0–2.0) ng/l, assuming nominal peak concentrations. Responses observed in the microcosms did not differ between system types and sampling dates, indicating that ecological threshold levels are not affected by different community structures, including taxa sensitive to TPT. Although confidence intervals largely overlap, the laboratory-derived invertebrate SSD curve was slightly less sensitive than the curves from the microcosms. Possible explanations for the more sensitive field response are delayed effects and/or additional chronic exposure via the food chain in the microcosms.

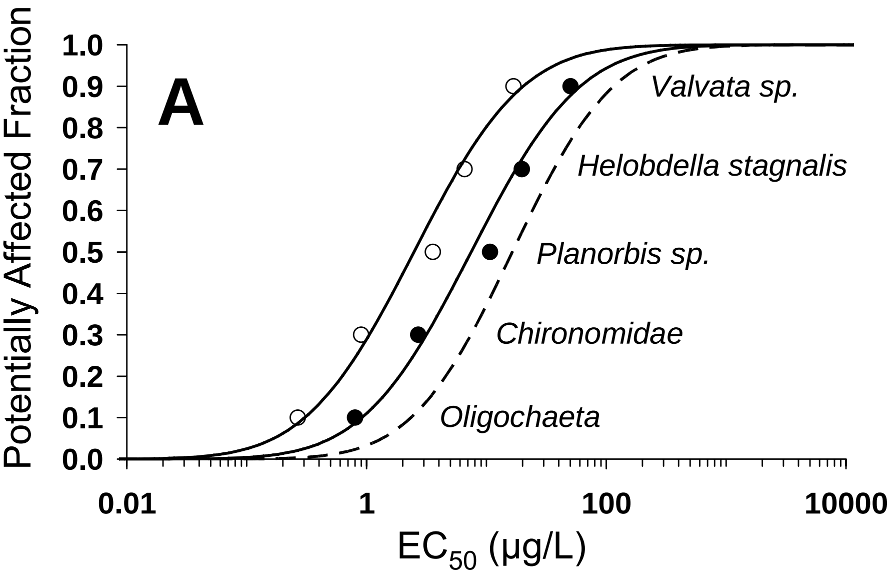

Figure 24. SSD curves of invertebrates after treatment with the fungicide TPT in outdoor cosms (constructed with clean sediment) based on initial nominal concentrations (•) and based on 21-days time weighted average concentrations (o). The figure presents the SSD curves at 2 weeks after application of TPT. The dashed line represents the 96 h-SSD curve of the invertebrates tested in the laboratory SST.

16.4 Implications and possible use

This paper illustrates that, SSD-derived threshold values are valuable for use in the risk assessment of fungicides. But it also shows that, for this compound (which accumulates in the food chain), data from conventional acute laboratory single species tests with invertebrates cannot be simply used to assess the risk to the aquatic community exposed to a similar concentration regime (single application) as simulated in our microcosm experiment. There is a need to consider the exposure conditions and/or endpoints affected. To address these concerns, more information is needed on the long-term, field effects of fungicides.

17 Using the expert model PERPEST to translate measured and predicted pesticide exposure data into ecological risks

By: P.J. van den Brink, C.D. Brown & I.G. Dubus

17.1 Problem definition

An important topic in the registration of pesticides and the interpretation of monitoring data is the estimation of the consequences of a certain concentration of a pesticide for the ecology of aquatic ecosystems. Solving this problem requires predictions of the expected response of the ecosystem to chemical stress. Until now, a dominant approach to make such a prediction is the use of simulation models or safety factors. The disadvantage of using safety factors is that this is a crude method that does not provide any insight into the concentration–response relationships at the ecosystem level. On the other hand, simulation models also have serious drawbacks; they are often very complex, lack transparency, their implementation is expensive and there may be a compilation of errors due to uncertainties in parameters and processes. In this paper, we present the expert model prediction of the ecological risks of pesticides (PERPEST) that overcomes these problems. It predicts the effects of a given concentration of a pesticide based on the outcome of already performed experiments using experimental ecosystems. This has the great advantage that the outcome is more realistic. The study is described in detail by Van den Brink et al. (2006b).

17.2 Approach

Case-based reasoning (CBR) is a problem-solving paradigm that is able to utilize the specific knowledge of previously experienced, concrete problem situations (cases) for solving new problems. CBR is an approach that enables incremental, sustained learning. New experience is retained, making it immediately available for future problems. A very important feature of case-based reasoning is its ability to learn. By adding present experience to the case base, improved predictions can be made in the future.

Wageningen University and Alterra have developed a Case Based Reasoning methodology for the prediction of pesticide effects on freshwater ecosystems (Van den Brink et al. 2002c). This methodology is called PERPEST (Prediction of the Ecological Risks of PESTicides) and is incorporated into a user-friendly interface (Van Nes and Van den Brink 2003). It predicts the effects of a certain concentration of a pesticide on various community endpoints simultaneously. The database containing the "experiences from the past" was constructed by performing a review of freshwater model ecosystem studies evaluating the effects of insecticides and herbicides (Brock et al. 2000a; Brock et al. 2000b). The PERPEST model searches for analogous situations in the database, using relevant environmental fate characteristics of the compound, exposure concentration and type of ecosystem to be evaluated. A prediction is made by using weighted averages of the effects reported in the most relevant literature references. PERPEST results in a prediction showing the probability of effects (no effect, slight effects or clear effects) on the various groups of endpoints, including structural and functional endpoints (see Figure 25). The PERPEST model is described in Van den Brink et al. (2002c) and is available at www.perpest.wur.nl.

17.3 Main results

This paper focuses on how measured and modelled concentrations can be translated into ecological risks using the PERPEST model. Figure 25 shows the prediction of risks on functional endpoints over a concentration range of atrazine. Risks of measured concentrations of atrazine can be read from the figure, with PERPEST also providing the option of obtaining confidence intervals. The paper also explains how a modelled concentration cumulative distributions function for ditches can be translated into Joint Probability Curves expressing the predicted frequency of exceeding a given initial exposure concentration in predicted ditches and the frequency that the concentration would result in clear effects on functional and structural endpoints. The paper also explains how this joint probability curve, which is difficult to interpret, can be translated into a single measure of risk: the overall frequency with which exposure of the predicted ditches would result in clear effects on functional and structural endpoints.

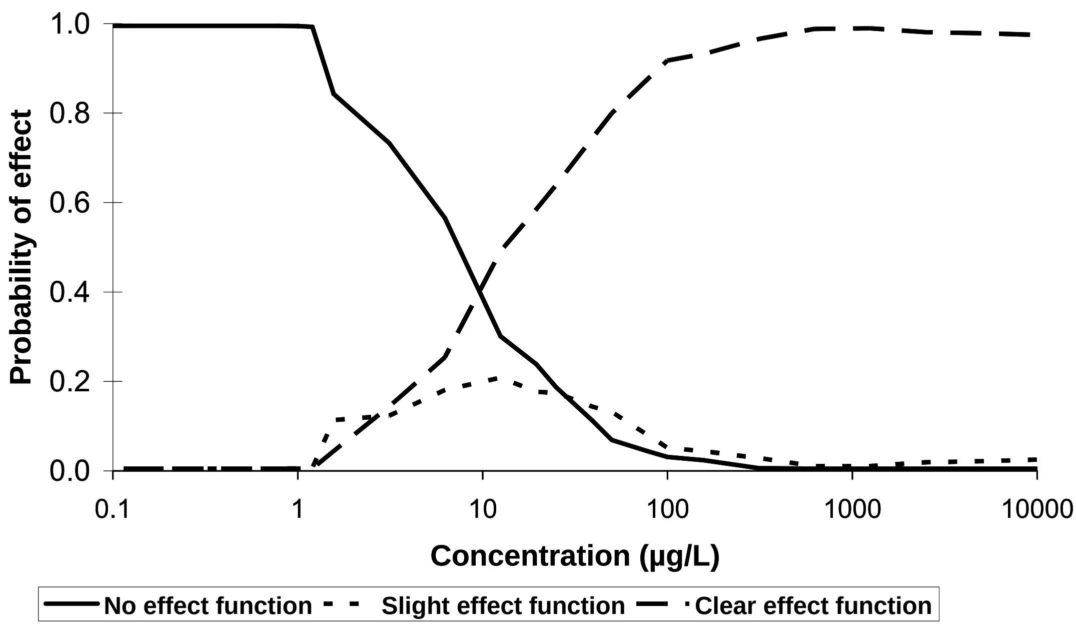

Figure 25. Relation between the probability of no effects (connected line), slight effects (line with short dashes) and clear effects (line with long dashes) on functional endpoints as predicted by the expert model PERPEST for atrazine over a concentration range.

17.4 Implications and possible use

A key advantage of PERPEST over single species/safety factor analyses is that it decreases the uncertainty of extrapolating to the ecosystem level. Since the effects data are based on mesocosm studies, there is still the uncertainty of extrapolating the mesocosm data to the field. The benefit of PERPEST is not only that it removes some uncertainty by including experience from other pesticides, but also that it quantifies the uncertainty by taking a probabilistic approach and providing uncertainty limits to predicted values.

Chemical monitoring is often performed to evaluate the quality of surface waters for regulatory purposes and/or to evaluate environmental status and trends. Such monitoring activities are expected to increase in Europe due to the Water Framework Directive, which was adopted on 23 October 2000 to establish a framework for the Community action in the field of water policy (EU 2000). For surface waters, this directive defines good ecological and chemical status, which must be monitored in a timely fashion. In this paper, we provide an example of how these monitoring data can be translated into ecological risks. It is often mixtures of pesticides, rather than single compounds, which are found to be present in surface waters. It would therefore be a great improvement if PERPEST could estimate the overall ecological risks associated with measured concentrations of different pesticides, i.e. mixtures of pesticides. This will be possible in the next version of PERPEST, to be released in the near future.

18 Detecting effects of "the grey veil" of pollutants on earthworm populations in river floodplains. Does flooding blur the picture?

By: C Klok

18.1 Problem definition

In industrialized countries, river floodplain soil can be severely polluted with heavy metals. However, published studies on the effect of heavy metal pollution on soil invertebrates, like earthworms, in such areas were inconclusive. This is unexpected since studies in other, less dynamic, environments showed clear effects at even lower levels of pollution. Pollutants are only one stress factor in floodplains that can influence earthworm populations. Flooding itself seems to have a major impact on earthworm populations by strongly increasing the variation in biomass and abundance. This increase in variation reduces the detectability of pollutant-induced changes in these parameters. In this paper, we specifically look at the impact of flooding on earthworm populations with the ultimate aim to disentangle pollutant from flooding effects and make the impact of the "grey veil" visible. Without insight in the role of flooding, the effects of the "grey veil" in floodplains are bound to remain unclear.

Field data showed that in frequently flooded sites, earthworms matured at a smaller weight, which implies a younger age compared to sites that were flooded less often. Seemingly, flooding not only has an impact on the abundance and biomass of earthworms, but also results in a change in their development. Flooding events may even kill all of the individuals of the earthworm species *Lumbricus rubellus* living in river floodplains, although earthworm cocoons usually survive immersion, permitting populations to recover after the flood waters recede. Yet, if the area is flooded again before earthworms hatching from cocoons or migrating from adjacent areas reach reproductive maturity, it is unlikely that their populations will recover. The objective of the present study is to determine the importance of the length of the dry period for population recovery in *L. rubellus*. The study is described in detail by Klok et al. (2006b).

18.2 Approach

Earthworms were collected at three floodplain sites along the Rhine River that were frequently, moderately or seldom flooded. Reproductively mature *L. rubellus* from the frequent flooded site were half the weight and probably younger than those from the other sites. A mechanistic population model (PODYRAS) was used to estimate the time for earthworm development from hatching to reproductive maturity, and to calculate the probability of population recovery after flooding.

18.3 Main results

The model results showed that the probability of population extinction increases when the dry period is not long enough for individuals to reach reproductive maturity. When this condition is met, population extinction is virtually absent due to the high lifetime reproductive output of *L. rubellus*.

Parameterization of the model with site-specific data indicated that population survival on the site with the shortest dry period drastically decreases if worms mature at the weight measured at the other sites.

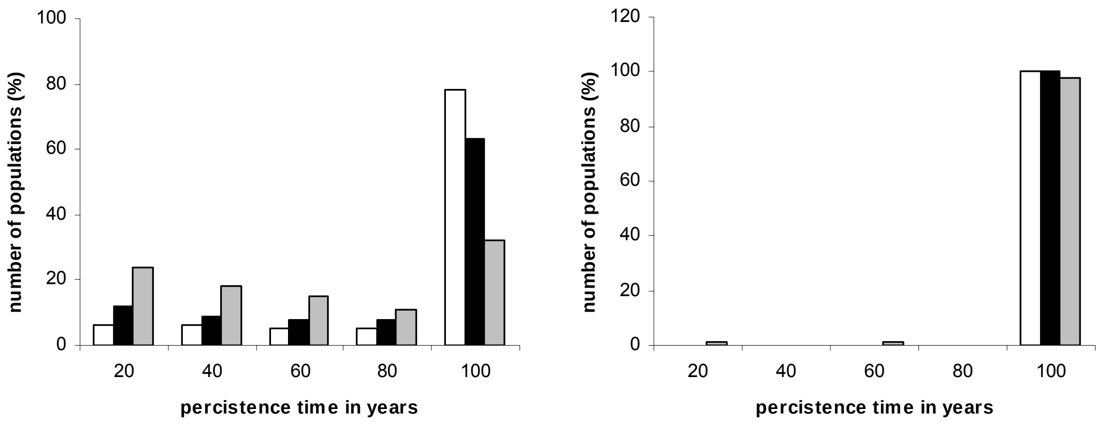

Figure 26. Frequency of the number of succeeding years that *L. rubellus* populations survive if extinctions only result from an excessively short dry period, so that individuals do not mature. Maturation based on adult weights measured in 2002 on site; Frequently (open bar), Moderately (grey bar) or Seldom inundated (dark bar). Populations live in a variable environment with dry periods randomly drawn from: (a) the distribution of dry periods within frequently-inundated sites, (b) the same distribution of dry periods in moderately-inundated sites.

18.4 Implication and possible use

The results strongly suggest that the duration of the dry period is critical for population recovery in river floodplains and that earthworm populations have adapted to local (site-specific) conditions. This adaptation to the local flooding regime may have a large influence on the earthworm dynamics in floodplains. Therefore, inundation may indeed mask the effects of pollutants on earthworm biomass and density.

19 Do earthworms (*Lumbricus rubellus*) adapt to flooding in wetlands by early maturation? Support from field data

By: C. Klok & N. Plum

19.1 Problem definition

The results in Chapter 18 show that the effects of pollution may be masked by flooding stress, since earthworms adapt to flooding by maturing at an earlier age. In this paper we investigate the extent of adaptation to flooding by earthworms. Adaptation to flooding by maturing earlier at a lower biomass has been suggested for *Lumbricus rubellus* (Hoffmeister 1843) (Klok et al. 2006b). Given the small number of sites used in the study of Klok et al. (2006b), however, it remains unclear how widespread this adaptation to flooding is in *L. rubellus*. In this paper we analyse an extensive database on earthworms in flooded grasslands to assess the generality of this form of adaptation. The study is described in detail by Klok and Plum (in press).

19.2 Approach

We used a large dataset based on 76 recent studies on terrestrial invertebrates in unpolluted wetlands to validate the results of a mechanistic population model (PODYRAS), which predicted adaptation by maturation at an early age as a consequence of flooding. From this dataset we selected all sites for which complete information on earthworm species composition, density and biomass was recorded. The influence of inundation intensity on earthworm parameters (abundance, total and individual weight) was tested with One-Way-ANOVAs (followed by Tukey tests for pair-wise comparisons). Statistical tests were performed for all data and for data grouped by soil type.

19.3 Main results

The results indicate that flooding indeed seems to decrease individual biomass, suggesting adaptation to flooding (Figure 27). Moreover, the results also indicate that the timing of sampling is of major importance to make the effects of flooding on individual biomass observable.

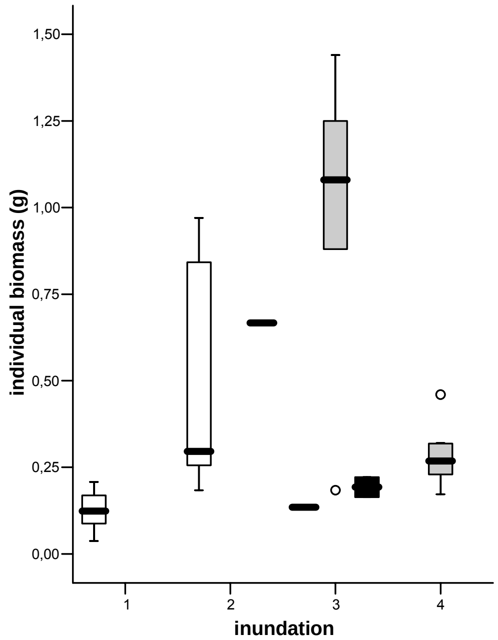

Figure 27. Mean individual earthworm weight (g) classified by soil type and inundation category. White: gley soil (n=15), grey: histosol (n=15), black: marsh soil (n=3). Cat.1: episodic, cat. 2: regular, cat. 3: extensive, cat. 4: waterlogged. Median values, interquantile range and outliers (circle).

19.4 Implications and use

The implications of this research, in combination with the results of Klok et al. (2006b), indicate that inventories of the impact of pollutants on earthworms in wetlands and floodplains should be interpreted with caution. Given the fact that adaptation to flooding is a widespread phenomenon, variation in earthworm data (abundance and biomass) may largely be the result of flooding stress, which adds noise to these parameters. Therefore to achieve statistical rigour on the relationship between pollutants and biomass and abundance, many replicates are needed in the test. Moreover information on flooding frequency is also needed to assess the interaction between pollution and flooding.

20 Combined effects of pollutants and inundation stress on earthworm populations in river floodplains

By: C. Klok

20.1 Problem definition

Given the large influence of flooding on earthworm populations as shown in Chapters 18 and 19, in this paper we hypothesize that the absence of clear, consistent effects in published studies might be due to the low level of replication in individual studies. To analyze effects of heavy metals on earthworms, we combined reported data from studies on river floodplains in the Netherlands and Belgium. The study is described in detail by Klok et al. (2007).

20.2 Approach

We selected published studies from the literature that reported heavy metal levels, earthworm data (species composition, biomass, density and individual weight) and flooding data. We analyzed the effects of heavy metals on earthworms and considered the interaction effects of stress and inundation.

20.3 Main results

The results (Figure 28) indicate clear effects of heavy metals on species composition, abundance, biomass and individual weight of earthworms, as well as interaction between copper and inundation. These results were not detected in the studies on which this paper is based, clearly showing that the level of replication was too low in these underlying studies.

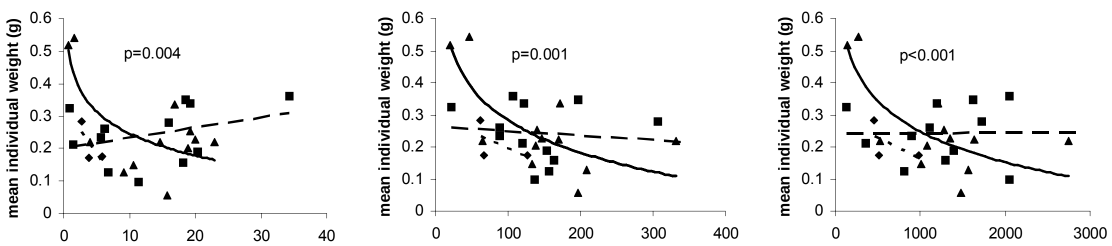

Figure 28. Relationship between total soil concentration of heavy metals and average individual biomass of earthworms for floodplain sites of the Rhine and Scheldt rivers. Triangles: seldom (S) inundated sites; squares: sites with moderate inundation (M); diamonds: sites with frequent inundation (F). Solid lines: regression lines for S sites; dashed lines: M sites; dotted lines: F sites. P values in graph are given for S regressions. Regression coefficients are significantly different for S and M for all metals (Cd (p=0.004), Cu (p=0.037) and Zn (p=0.013)).

20.4 Implications and possible use

This study shows that in dynamic environments such as river floodplains, the effects of heavy metals on earthworm biomass and density are difficult to detect. Given the increased variation in abundance and density data as a result of flooding, the number of replications of field inventories should be high to detect effects. This is an important result, since it implies that heavy metals do have clear detrimental effects which are only detectable in large studies due to the high variability in the data induced by other stress factors. This result is in contrast with the general opinion that heavy metals have only minor effects in floodplains as a result of their low bioavailability.

21 Impact of climate change and floodplain rehabilitation on terrestrial earthworm food chains

By: I. Thonon & C. Klok

21.1 Problem definition

River floodplains are dynamic and fertile ecosystems where soil invertebrates such as earthworms can reach high population densities. Earthworms are an important food source for a wide range of organisms, including protected species. Flooding diminishes earthworm numbers. Populations recover from cocoons that survive floods. If the period between two floods is too short for cocoons to develop into reproductive adults, populations cannot sustain themselves. Both climate change and floodplain rehabilitation change the flooding frequency affecting earthworm populations. The present paper estimates the influence of climate change and river rehabilitation on the viability of earthworm populations in a Dutch floodplain along the lower Rhine river. Moreover, the possible impact of changes in the availability of pollutants due to both climate change and rehabilitation and the consequences on earthworm populations and their predators are discussed. The study is described in detail by Thonon and Klok (2007).

21.2 Approach

We selected the regularly flooded Afferdensche and Deestsche Waarden (ADW) floodplain along the lower Rhine river as a study area. The Rhine river has an average discharge of 2250 $m^3\ s^{-1}$ at the Dutch-German border. In the Netherlands, the river splits into the Waal, IJssel and Nederrijn rivers. The ADW floodplain is located along the Waal River branch (average discharge: 1500 $m^3\ s^{-1}$). We calculated the flooding frequency for this floodplain based on climate change scenarios and rehabilitation plans. We then used earthworm data (for the species *Lumbricus rubellus*) and the model PODYRAS to assess the impact of the changed flooding frequency on earthworm population viability. We discussed the effects of heavy metal stress on earthworms as a result of changes in heavy metal availability resulting from climate change and river rehabilitation.

21.3 Main results

The results show that climate change has almost no adverse effect on earthworm viability. This is because climate change reduces the flooding frequency during the earthworms' growing season. Floodplain rehabilitation, on the other hand, reduces the part of the floodplain area where populations can sustain themselves. Before rehabilitation, only 12% of the floodplain area cannot sustain a viable earthworm population. After rehabilitation, this increases to 59%, 28% of which is due to more frequent flooding (Figure 29). Enhanced exposure to soil contaminants may further suppress earthworm viability. This could frustrate further nature development and the introduction of earthworm-dependent species such as the badger (*Meles meles*) or little owl (*Athene noctua vidalli* species), two objectives of the river rehabilitation plans in the Netherlands.

Figure 29. Viability map for *Lumbricus rubellus* in the present (a) and future (b) ADW floodplain, with the present topography used as background.

21.4 Implications and possible use

River floodplains are an important foraging habitat for earthworm predators like the badger *Meles meles* and the little owl *Athene noctua vidalli*, species that have a protected status in the Netherlands. For these species, floodplain rehabilitation plans may greatly reduce the amount of high quality foraging habitat as a result of low availability of their food (earthworms). In addition, these predators may suffer from secondary poisoning, given the fact that surviving earthworms may contain higher pollutant levels (Van Vliet et al. 2005).

22 Impact of the “grey veil” of sediment-bound contaminants on macro-invertebrate communities in the Rhine-Meuse delta

By: E.T.H.M. Peeters, C. van Griethuysen, H.J. De Lange & A.A. Koelmans

22.1 Problem definition

Most sediments in the Netherlands are moderately polluted with trace metals, polycyclic aromatic hydrocarbons (PAH), polychlorobiphenyls (PCBs) and other organic chemicals such as flame retardants, organotin-compounds and dioxins. The negative effects of these toxicants as measured in single-substance/single-species toxicity tests are well-documented. An important SSEO-question, however, is to what extent these contaminant mixtures pose a threat to benthic communities. These communities are largely made up of macroinvertebrates, which are the key to the benthic-pelagic coupling in aquatic ecosystems. They pass on carbon (food) to higher trophic levels and determine the carrying capacity for many predators, such as fish and birds. Is it possible to relate endpoints like macroinvertebrate species diversity and abundance to toxicant concentrations in sediment? Relevant questions and tasks from the SSEO perspective are:

(i) whether it is possible to detect contaminant effects,
(ii) to quantify the relative importance of different contaminant groups
(iii) to quantify the relative importance of contaminant stress to natural stressors, and
(iv) to derive sediment quality criteria from these findings..

Under field conditions, dose-effect relationships may be highly variable due to adaptation of organisms, variation of contaminant bioavailability, variation of habitat and stressors other than toxicants. For invertebrates, “non-toxic” stress factors nay include suboptimal flow, substratum, temperature, salinity, light (UV), food quality, diet and predation. These factors are all known to yield causal effects. Yet, under field conditions, their relative importance is not known for individual location nor between locations. Furthermore, other unknown factors still may affect the endpoints. In summary, macroinvertebrates communities reside in a highly multivariate environment.

22.2 Approach

The above issues can be addressed using Canonical Correspondence Analysis (CCA) combined with partitioning of the variance (Ter Braak and Verdonschot 1995). Between 2000 and 2004, four studies were published in which this procedure was used to separate contaminant effects from ecological (habitat) effects in aquatic ecosystems in the Netherlands. For details on this procedure, see Peeters et al. (2000). The approach used in these studies is briefly outlined below.

The procedure first detects the total variance in the species dataset, and subsequently determines up to what percentage a given explanatory variable (or group explanatory variables) can explain that total. As used here, “explains” refers to the existence of a statistical correlation between that part of the variance and that explanatory variable. The significance of these correlations was assessed by Monte Carlo permutation tests (Peeters et al. 2001). The procedure also allows the calculation of a percentage of “unexplained variation”. This percentage covers the unknown explanatory variables, the effects of measurement error, variation due to uncertainties in exposure, variation due to mixture toxicity effects (antagonism, synergism), biological variation, and other variation in dose-effect relationships. Part of this variation stems from differences between the investigated sites. However, the separate quantification of the total unexplained variation guarantees that the percentages of explained variation are relatively insensitive to these uncertainties. If potentially explanatory variables were correlated (co-variance of variables), only one of them was used. Consequently, the remaining variable then accounted for the group represented by that single variable. Detection of co-variation was done by univariate correlation analysis and by calculating variance inflation factors as part of CCA. Still, if some part of the explained variance could not be attributed to a single explanatory factor, that part was attributed to multiple variables and reported as “shared variance”.

Furthermore, the detection of significant percentages of explained variances further may be limited by two problems related to exposure under field conditions. The first problem is that total contaminant concentrations are rivers usually known, whereas only available fractions pose negative effects. The magnitude of these bio-available fractions may vary considerably among chemicals and locations and thus should be accounted for. This problem is circumvented by using measured or estimated bio-available fractions as input to the CCA. Bioavailability is measured by mild extraction with Tenax beads (for organic chemicals), or by mild acid extraction with acid followed by a correction for sulphidic fixation of metals (Simultaneously Extracted Metal – Acid Volatile Sulphide; AVS-SEM concept). In other studies, we approximated available fractions by normalizing total concentrations to either organic matter (OM) or clay content. PCBs and PAHs, for instance, reside in the OM fraction, thus OM normalized concentrations mimic their concentration in OM. The latter concentration often is proportional to the pore-water concentrations the organism is exposed to.

The second problem is that toxicant levels and species abundance/diversity are usually measured at the same time, whereas the latter also are influenced by previous exposure. However, the relevant exposure time frame for macro-invertebrates would be less than one year. For each year, “background” contaminants typically show negligible time trends, so that changes in exposure may be considered negligible.

In the statistical procedures, different alternatives with respect to variable selection or bioavailability assessment can be tested. The combination of variables that yields the largest percentage of explained variance and the lowest percentage of shared variance can be considered as best. Sometimes, statistical methods to detect effects are criticized for their limited potential to detect causal relationships. In our opinion, this is not a serious problem. First, detection of in situ effects implies that the system is not treated or manipulated in any way. Any treatment in a field experimental setup would introduce artefacts. In other words, there is no alternative. Second, if variance partitioning eventually detects a significant correlation between a toxicant concentration and particular species abundance, the only question is whether the toxicant caused that abundance, or the reverse. The latter option only is relevant if macro-invertebrates are able to disperse or degrade toxicants to lower concentration levels. In most settings, this is not a plausible option.

22.3 Main results

The procedure described in the previous section was applied to datasets obtained from:

1. the North Sea Canal (Van Griethuysen et al. 2004),
2. the Rhine-Meuse Delta 1992-1995 (SSEO site Brabantsche Biesbosch, Dordtsche Biesbosch and Hollands Diep, Haringvliet, Nieuwe Merwede (Peeters et al. 2000)),

3. ten floodplain lakes along the IJssel and Waal rivers (including the SSEO site Afferdensche and Deestsche Waarden), and
4. the Nederrijn (Peeters et al. 2001)) and again the Rhine Meuse Delta in 2001 (SSEO site Brabantsche Biesbosch, Dordtsche Biesbosch and Sliedrechtse Biesbosch).

The results are briefly reviewed below.

Impact of trace metals in the North Sea Canal, the Netherlands (Van Griethuysen et al. 2004)
Macroinvertebrates were studied along a salinity gradient in the North Sea Canal to quantify the effect of trace metals (cadmium, copper, lead, zinc) on community composition. In addition, two methods for assessing metal bioavailability (normalizing metal concentrations on organic carbon and on the smallest sediment fraction) were compared. Factor analyses showed that normalizing trace metals resulted in an improved separation of trace metals from ecological factors (depth, organic carbon, granulometry and chloride). The variation in the macroinvertebrate data was partitioned into four sources using partial canonical correspondence analysis, with the partitions being purely ecological factors, purely trace metals, mutual ecological factors and trace metals, and unexplained. Partial canonical correspondence analysis applied to total and normalized trace metal concentrations gave similar results in terms of unexplained variances. However, normalization on organic carbon resulted in the highest percentage of variation explained by purely ecological factors and purely trace metals. Accounting for bioavailability thus improves the identification of factors affecting the in situ community structure. Ecological factors explained 45.4% of the variation in the macroinvertebrate community composition in the ecosystem of the North Sea Canal, and trace metals explained 8.6%. These contributions were significant, and we concluded that trace metals significantly affected the community composition in an environment with multiple stressors. Variance partitioning is recommended for incorporation in further risk assessment studies.

Impact of trace metals, PAHs, oil and PCBs in the Rhine-Meuse delta (De Lange et al. 2004).
Between 1992 and 1995, data were collected in the enclosed Rhine-Meuse delta (in the Netherlands) on macroinvertebrates, sediment contaminant concentrations and ecological factors. The effects of various groups of pollutants (polycyclic aromatic hydrocarbons, trace metals, oil, polychlorinated biphenyls) and ecological variables on the structure of the macroinvertebrate community were quantified. Ecological factors explained 15.6-16.8% of the macroinvertebrate variation, while PAHs explained 12-14.9%, trace metals explained 13.1 – 15.4%, oil 0 – 1.6 % and PCBs 0 – 2.4%. Another 11.9 - 24.8% was explained by the covariation between ecological variables and contaminants. Polycyclic aromatic hydrocarbons explained a larger part of the variation than trace metals. The contributions of oil and polychlorinated biphenyls were small but still significant. Elevated contaminant concentrations were significantly associated with differences in the macroinvertebrate food web structure.

Impact of trace metals in floodplain lakes along the river IJssel, Waal (including ADW) and Nederrijn (Van Griethuysen et al. 2004)
We analyzed the in situ effects of trace metals and common environmental variables on benthic macroinvertebrate communities in floodplain lakes. Ten lakes were selected: three lakes along the IJssel, four lakes along the Waal (SSEO at the location of the Afferdensche and Deestsche Waarden, ADW), and three lakes along the Nederrijn. Alternative measures of trace metal availability were evaluated, including total metals, metals normalized on organic carbon (OC) or clay, simultaneously extracted metals (SEM), combinations of SEM and acid-volatile sulphide (AVS), and metals accumulated by detritivore invertebrates (Oligochaeta).

Sixty-eight percent of the variation in benthic community composition was explained by a combination of 11 environmental variables, including sediment, water, and morphological characteristics with trace metals. Metals explained 2 to 6% of the community composition when SEM − AVS or individual SEM concentrations were considered. In contrast, total, normalized, and accumulated metals were not significantly linked to community composition. We concluded that accounting for bioavailability, for instance (for metals) by assessing SEM or SEM − AVS concentrations is useful for risk assessment of trace metals on the community level.

Impact of trace metals, PCBs and PAHs in Brabantsche Biesbosch, Dordtsche Biesbosch and Sliedrechtse Biesbosch (De Lange et al. 2004)
The effects of sediment-bound contaminants on the communities of benthic macroinvertebrates are unclear. Sixteen locations along a pollution gradient were investigated in creeks in the Biesbosch floodplain area. Sediment samples were analyzed for bulk sediment characteristics and contaminants (total and bioavailable concentrations of trace metals, PAHs and PCBs). Metal availability was assessed by SEM-AVS measurements. PCB/PAH available concentrations were determined by mild extraction with Tenax beads. Macroinvertebrates were sampled and identified to species level. Benthic macroinvertebrate species richness was negatively affected by sediment contamination. CCA with variance partitioning revealed that "ecological" factors (habitat) explained 33.3% of the community composition, while PAHs explained 2.8%, PCBs explained 4.3% and trace metals explained 5.3% of community composition. Finally, 5.6% related to shared variance and the rest remained unexplained (either unexplained habitat or contaminant factors). The effect of predation on benthic community structure was also assessed in multivariate analysis. A comparison between bottom-up (habitat and contaminants) and top-down (predation) effects showed that bottom–up control was more important than top–down control in our systems. Thus, the chronic effect of sublethal concentrations of sediment-bound contaminants on the benthic communities was stronger than predation.

22.4 Implications and possible use

The results show that the "grey veil" of sediment-bound contaminants significantly alters community composition. Roughly speaking, it appears that about 10-30% of community composition is explained by these contaminants. These ranges may be considered as minimum estimates, since several contaminant classes were not included and could have explained an additional part of the unexplained variance in these studies. The order of magnitude also is similar to that which is found in similar environments (Pinel-Alloul et al. 1996). From an ecological perspective, these results mean that contaminants are important, but may not be the dominating mechanism in structuring ecosystem type or ecological processes. From the perspective of environmental protection, however, preserving specific species or diversity targets (for instance preserving >95% of species in an ecosystem) are legitimate goals. From that perspective, and given the fact that part of the contaminant effects have been unaccounted for, these impacts on aquatic communities may be considered considerable and may warrant further investigation.

The possible use of the methodology described in this chapter is fourfold:

- First, the procedure can be used as such. That is, the same procedure can be used in triad-like approaches to assess the relative impact of particular contaminant classes on macro-invertebrate communities.
- Second, the impacts detected with this method may be compared with other models claiming to detect ecological effects. The question and challenge, for instance, is to link the detected field effects to inferences made by species sensitivity distribution (SSD) model predictions.

- Third, the datasets obtained from this work can be used as input for case-based reasoning models, which constitute an alternative methodology to link community effects to environmental variables such as habitat characteristics and contaminant concentrations (Van den Brink et al. 2005).
- Fourth, combined datasets may be used to derive field-based SSDs, which subsequently can be compared to current sediment quality guidelines (Leung et al. 2005).

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Section 3. Selected SSEO studies not involving validation of models

The following set of chapters provides an extension of the set of studies in Section 2 by presenting selected studies in which the findings provide a broader view of the results of the SSEO programme. Looking only into the set of studies with a component of model validation would result in a biased impression on the breadth of SSEO programme results. This means that the results of the three sampling sites are relevant for the Dutch situation, but they may not represent the full array of possible responses to diffuse exposure to toxicant mixtures. Due to practical requirements, the selected samples all involve contributions that were provided by researchers working in the integration project, but who were also collaborating with other teams, and contributions from site coordinators.

23 Effects of heavy metals on the structure and functioning of detritivores in the contaminated floodplain area in the Biesbosch, the Netherlands

By: P.H.F. Hobbelen, P.J. van den Brink, J.F. Hobbelen & C.A.M. van Gestel

23.1 Problem definition

The aim of this study was to determine the effects of heavy metal pollution on the structure and functioning of detritivore soil communities, which consist of isopods, millipedes and earthworms, in 15 heavily-polluted floodplains located in the delta area of the Rhine and Meuse rivers in the Netherlands. The 15 study sites represent a gradient in Zn, Cu and Cd concentrations. For more details see Hobbelen et al. (2006c).

23.2 Approach

The structural attributes of the detritivore community that were assessed were the species richness and densities in the field sites. The functioning of the detritivore community was studied by determining organic matter decomposition using litter bags and feeding activity with the bait lamina method. Concentrations of Cd, Cu and Zn were measured in soil, pore water and 0.01M $CaCl_2$ extracts of the soil, adult earthworms and plant leaves.

23.3 Main results

Results show that metal pollution is not a dominating factor determining the species richness and densities of the selected detritivore groups. The biomass of the earthworm *Lumbricus rubellus* was positively and significantly correlated to Zn concentrations in pore water and 0.01M $CaCl_2$ extracts. Litter decomposition was significantly and positively correlated to detritivore biomass and 0.01M$CaCl_2$ extractable Cd concentrations in soil and negatively to pH-$CaCl_2$, although the range of pH values was very small.

23.4 Implication and possible use

It can be concluded that in spite of high metal levels in the soil, bioavailable concentrations are too low to result in clear negative effects on the structure and functioning of detritivores in the Biesbosch.

24 Metals affect secondary stress sensitivity of nematode field populations

By: A.W.G. van der Wurff, S.A.E. Kools, M.E.Y. Boivin, P.J. van den Brink, H.H.M. van Megen, J. Riksen, A. Doroszuk & J.E. Kammenga

24.1 Problem definition

The effects of exposure of soil communities to toxicants are well documented. Soil community structure is determined by tolerance, adaptation and species loss. The species that are the first to become extinct are those that have a long generation time, few offspring and other characteristics that render them sensitive. These species are known in ecological theory as K-strategists. Many proposed indices use this theory of K- versus *r*-strategists, originally proposed by Odum in the context of succession to evaluate effects of disturbances on soil community composition. The theoretical framework implicitly assumes that various kinds of disturbance will eventually result in similar types of ecological effects. Although this approach is of value, the kind of disturbance may also trigger a specific response.

In addition, it has frequently been hypothesized that stressors may alter the possibilities of populations or communities to respond to other, i.e. secondary, stressors. Soil communities may become adapted to a wide range of disturbances as a result to exposure to a contaminant. Although experimental evidence of co-tolerance to multiple stressors has been reported, the magnitude of this phenomenon and the role of the type of disturbance are unclear.

The study aimed to reveal whether complexity, specifically community and trophic structure, of chronically stressed soil systems is at increased risk or remains stable when confronted with a subsequent disturbance, in this case a heat shock or exposure to a contaminant.

24.2 Approach

We focused on grassland with a history of 400 years of patchy contamination. The long-term history of contamination is argued to be important to monitor an ecologically relevant response (see Ramsey et al., 2005). Nematodes were used as model organisms because they are an abundant and trophically diverse group and are representative of the soil food web and ecosystem complexity. In a field survey, a relation between contaminants and community structures was established. After this, , two groups of intact soil columns (i.e., mesocosms or Terrestrial Model Ecosystems) that differed in contamination background (high contaminated versus low contaminated) were exposed to different disturbance regimes, in this case the contaminant zinc or a heat shock. Community-level effect parameters were studied to determine whether the differently pre-exposed communities differed with respect to secondary stress exposures. The original study is described by Van der Wurff et al. (2006).

24.3 Main results

The zinc treatment of the soil columns revealed that community structure was stable, irrespective of background contamination levels. The amount of zinc that was used in our study was shown earlier to induce drastic changes in communities that were not pre-exposed. The absence of effects in our study implies that centuries of exposure to contamination led to adaptation of the soil nematode community irrespective of the spatial scale of contamination (on a scale of metres (Bosveld et al., 2000)). In contrast, the heat shock had adverse effects on species richness only in the highly contaminated soils (Figure 30). Therefore, our results show strong support for a hypothesis proposed earlier (Sankaran and McNaughton, 1999): that the history of contamination and the type of disturbance are important determinants of the response of communities. Although centuries of exposure to contaminants induced adaptation, the communities at the higher contaminated spots turned out to be more sensitive to another disturbance.

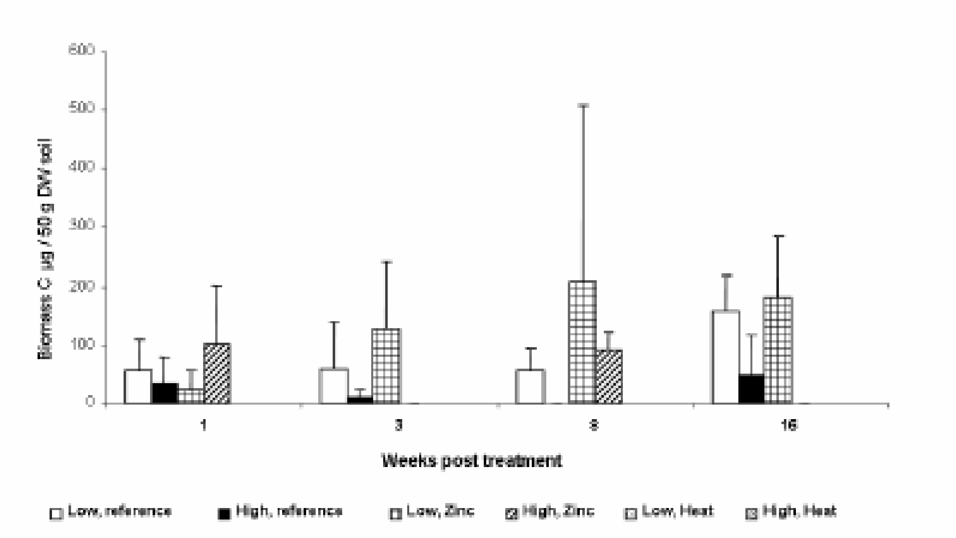

Figure 30. Total biomass of carnivorous nematodes in µg per 50 g dry weight soil in 730 reference and treated mesocosms from Low and High contaminated sites throughout 16 weeks. Bars represent averages (n = 3) with standard deviations. Carnivorous nematodes were present at the start of the experiment at week 0 in all mesocosms (not indicated).

24.4 Implication and possible use

Biodiversity can be affected in highly contaminated sites, but also at low exposures.The loss of higher trophic levels from the entire system, such as represented by carnivorous nematodes after the heat shock (Fig 22), accompanied with local biodiversity loss at highly contaminated sites appears to have resulted in detrimental effects on ecosystem functions.
The patchiness of the contamination in the grassland ecosystem may favour a rescue of ecosystem functions by means of re-colonization of the highly contaminated spots (spatial insurance hypothesis (Loreau et al., 2003)). However, it has been shown that species that occupy higher trophic levels have a large impact on ecosystem functioning. An elimination of top-down control, for instance, decreases grazer diversity, which in its turn may limit production (see also Duffy et al., 2005; Ebenman and Jonsson, 2005; Elmqvist et al., 2003). Therefore, our results show that contaminated systems are at risk, and this leads to the need for research towards effects of secondary stress, such as climate change, on disturbed ecosystems.

A comparison of two types of analyses, based on *r*-K features and Principle Component Analyses, revealed that *r*-K features do not correspond with the sensitivity of diverse groups of nematodes to stress. An example is the elimination of *rhabditians* after the heat shock in the high-contaminated soil columns. These opportunistic bacterivores are generally regarded as typical *r*-strategists. Indirect effects, such as horizontal, bottom-up and top-down mechanisms, may be responsible and are therefore crucial to take into account when studying effects on communities. Another interesting finding was that density compensation occurred rapidly, i.e. tolerant species quickly replaced sensitive species. This suggests that biomass is not a good indicator in stress-response studies.

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25 Pollution-Induced Community Tolerance as an ecotoxicological model to demonstrate effects?

By: M. Rutgers, M., P. van Beelen, M.E.Y. Boivin, J. Wittebol & L. Posthuma

25.1 Problem definition

Demonstration of ecological effects of contamination in the field situation is often hampered by the lack of a useful reference situation (sample or location). An ideal reference is completely identical to the contaminated situation, except for the contamination levels. Real ecosystems are highly dynamic and unique at large and very small scales. Therefore, natural fluctuations and factors that co-vary with the contamination level severely impede the demonstration of field effects of contaminants. In fact, this also explains why traditional ecotoxicology and risk assessment is based on chemical methodologies. The issue of a lack of useful reference sites is most pronounced when a “grey veil” of contaminants is present (rather than extreme levels of exposure), due to the small contaminant gradients.

In the late 1980s, Pollution-Induced Community Tolerance (PICT) was proposed as a new tool for demonstrating the field effects of contaminants and for ecological risk assessment (Blanck et al. 1988). PICT has been applied in aquatic ecosystems and later on also in terrestrial ecosystems (reviews: Blanck 2002; Boivin 2005; Boivin et al. 2002). PICT was considered as a solution to the problem of a lack of reference sites. The concept of PICT is based on the phenomenon that communities in an ecosystem exhibit increased tolerance as a result of exposure to the contamination. This effect can be deduced from artificial exposure experiments in the laboratory with field samples containing the exposed communities. In a sense, the “inherited” level of tolerance to a compound that is shown in artificial exposure experiments is considered to reflect the presence of a toxic level of exposure to the compound in the “parent” situation of the sampled community in the field. Hence, PICT is a model for identification of exposure in the field that has reached an effective, toxic level.

Several issues need to be addressed for a proper positioning of PICT in ecotoxicological research and ecological risk assessment:

1. Although community tolerance is plausibly the result of effective toxic exposure to that specific contaminant, co-tolerance phenomena and the induction of tolerance by other environmental factors (e.g. pH) also have to be considered.
2. Via validation studies it has to be demonstrated that PICT coincides with effects at the ecosystem level, e.g. on species densities, community composition and/or ecosystem functioning.
3. The issue of scale must be solved; it should be completely clear how PICT should be addressed in terms of the level of ecosystem impairment (at a level comparable with the application of SSDs in risk assessment).

25.2 Approach

In the framework of SSEO, the feasibility of PICT was tested with microbial communities and to a smaller extent with nematode communities. With the larger organisms, PICT becomes unpractical as a tool for risk assessment (Rutgers and Breure 1999). A literature review of PICT was conducted for terrestrial ecosystems (Boivin et al. 2002). Due to a complete lack of gradients in the aquatic ecosystem of the Demmerik, experiments with copper were performed in the laboratory. For the terrestrial ecosystems and the floodplains, PICT was addressed for lead and zinc.

25.3 Main results

Boivin et al. (2002) demonstrated a functional recovery of aquatic bacterial communities in biofilms after copper exposure had been removed from the system (Figure 31). Copper exposure first induced PICT, which coincided with a functional and a structural community response. After removal of copper from the system, recovery was demonstrated for PICT and functional characteristics of the biofilm community, but a very much smaller recovery was demonstrated for the structural parameters (DNA composition of the community). Consequently, recovery of functional community characteristics is possible, but structural community recovery is a much slower process.

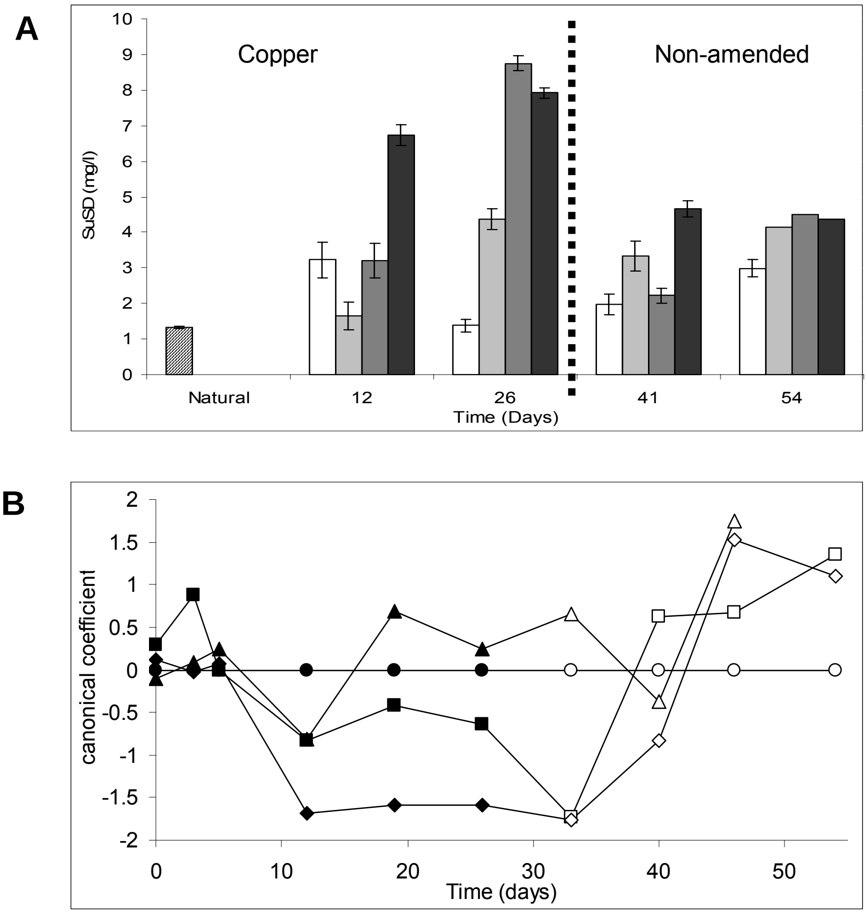

Figure 31. Results of experiments with copper exposure to aquatic biofilms in different aquaria. A. The median of substrate utilization sensitivity distribution (α SuSD mg/l) of different aquatic bacterial communities from biofilm exposed to different copper concentrations. 0 µM Cu: white bars, 1 µM Cu: light grey bars, 3 µM Cu: dark grey bars and 10 µM Cu: black bars. The error bars represent a 95 % confidence interval. B. Principal response curves (PRC) of community-level physiological profiles (CLPP) of aquatic bacterial communities from several biofilms. From day 0 until day 26, the biofilms were exposed to different copper concentrations, and

from day 26 until 54 all the aquaria were refreshed with ditch water without copper addition. Closed symbols represent the exposure period. Open symbols represent the recovery period. Copper treatments: 0 µM Cu: circles (•), 1 µM Cu: triangles (▲), 3 µM Cu: squares (■) and 10 µM Cu: diamonds (♦). Results are reproduced from Boivin et al. (2006a).

PICT for lead and zinc was not unequivocally demonstrated in terrestrial ecosystems of the Demmerik polder (Boivin et al. 2006a) despite reasonable contaminant gradients. Recently, J. Wittebol (data not shown) was able to demonstrate PICT for lead in samples of the Demmerik polder. These conflicting results might be caused by unknown exposure conditions to contaminants, making a proper exposure assessment more difficult. Van Beelen et al. (2004) performed a range of PICT studies with different contaminants and field sites. The general message from these and other studies reported in the literature was that PICT is a feasible instrument for use in retrospective ecological risk assessments in many instances.

25.4 Implications and possible use

PICT could be an interesting tool and model for ecological risk assessment in cases where natural variations and dynamics and co-correlations with environmental factors block a proper demonstration of field effects. Previously, we showed a way to view results from ecological observations, like the PICT response, on a scale which is relevant for ecological risk assessment (Boivin 2005; Rutgers and Den Besten 2005). However, PICT appears to be too complicated for a routine assessment, unless practical methods become available.

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26 Analysis of compiled microbial functional traits

By: C. Mulder, F. Kuenen, D. de Zwart, M. Rutgers & L. Posthuma

26.1 Problem definition

Although soil bacteria are known as the crucial element of ecosystem functioning, they remain neglected in most ecological risk assessments, both as bioindicators and as non-target organisms. This is rather surprising, in view of the wide variation in agro-ecosystems that arises from several bacterial-driven processes (Mulder 2006; Mulder et al. 2005b). In the present study, we compiled functional response data from various experiments with chronically exposed soil microbiota, as conducted both within and outside the SSEO programme, and analysed that data to investigate possible functional effects of chronic contaminant exposures in the field. The study results were interpreted in comparison to the results of a recent study, where short term influences of a single clear stress factor prevailed. The study data were mainly obtained from the SSEO studies of Boivin (2005).

26.2 Approach

Soil samples for the present study were collected from contaminated grassland field sites in the Netherlands. These sites are highly heterogeneous, and contain an array of contaminants, mostly heavy metals. Metal concentrations locally exceed the target value and the intervention value, suggesting an array of low to high pollution samples. Bacterial communities were isolated from each sample and treated to allow the ecophysiological profiles of the local bacterial communities to be studied. So-called Community-Level Physiological Profiles (CLPP) of the investigated bacterial samples were made after substrate-breakdown measurements in multiwell plates of BIOLOG Inc. (Hayward, CA, USA). These multiwell plates are marketed as EcoPlates and are specifically designed for microecological (metabolic activity) studies. EcoPlates contain three replicate sets of 31 different carbon sources in addition to a nutrient and salt solution and a redox dye. The plates are inoculated with 100 µl suspension per well and diluted (in a buffer solution). Bacterial suspensions were serially diluted from 3^{-1} until 3^{-12}. These dilutions were used to inoculate four EcoPlates per sample (Rutgers 2006). The CLPP was determined for frozen samples from the field (Boivin 2005) and for a series of microcosms under strictly controlled conditions, according to the step-by-step procedure:

1. Integrate well colour over time (WCD: one substrate; AWCD: average substrates)
2. Plot WCD as a function of bacterial dilution
3. Fit logistic curve to dilution-response data
4. Repeat steps 1 to 3 for all the other 30 substrates
5. Estimate the bacterial dilution corresponding to 50 % response for each substrate
6. Estimate average bacterial dilution corresponding to 50 % response
7. The resulting difference, per well, between 5 and 6, is considered as a measure of the microbial metabolic activity of that respective community.

26.3 Main results

Strong empirical evidence is available to show a different utilization of carbon compounds by soil bacteria according to environmental conditions but, in contrast to our expectation, comparable physiological evidence was lacking for the concentrations of heavy metals in SSEO sites such as the floodplains and the Biesbosch. As stated in Boivin (2006a), the effects of environmental contaminants are often confounded by natural variation and ecological stress factors, although the effects of natural factors seem to become more apparent when the pollution gradient is small, and the environment more dynamic.

Analyses of data from dynamic sediments of the SSEO-sites Biesbosch and floodplains over different years demonstrated that the functional differences between sites were dominated by the factor sampling area (Biesbosch versus floodplains), rather than by the contamination gradient ($p > 0.1$) or the site within the sampling area ($p > 0.05$). Apparently, the combination of high concentrations of heavy metals, flooding and variable soil acidity across these sampling areas seems to obscure the actual diversity of the bacterial ecophysiological traits. There are two dominant effects, yielding the location and the sampling year as dominant predictors of functional differences uncovered by CLPPs.

For soil pollution, soil parameters (organic matter, clay, humidity, pH) often confound the relationship between ecological effects and pollution. Boivin's PhD study aimed to assess the effects of heavy metals on bacterial communities in grassland soil and to discriminate these effects from natural variability. Organic matter content and many other environmental parameters showed correlations with metals. Surprisingly, total organic matter was negatively correlated to metal concentrations, demonstrating exclusive characteristics of this polluted site. Consequently, straightforward measurement of the metal effects was not possible. For the grasslands in the Demmerikse polder, Boivin concluded that heavy metals must have had an effect on bacterial communities, although the effects could only be filtered from the total variation by multivariate analysis of the CLPPs of a large number of different soil samples. This may seem artefactual at first sight, given the high exceedance of risk limits that characterizes this grassland site, but the metal gradients were still relatively small, and good quality reference locations were not available. In fact, any CLPP analysis is reproducible and highly discriminative between bacterial communities; despite the small metal gradient, the CLPP responses in the field from the Demmerikse Polder did show a recognizable ecotoxicological pattern.

However, the information that can be extracted from microbial communities in microcosms has important implications for the ecological risk assessment of environmental perturbations. For instance, some results showed an evident response of soil bacteria to the addition of plant residues of different kinds (see Mulder et al. 2006) in a microcosm test design (soil with and without straw addition). The high correlation between microbial activity (soil respiration) and metabolic fingerprints (CLPP traits) in that study showed not only a different degree of metabolization in different substrate guilds (carbohydrates, amino acids etc.), but also a high reproducibility of the microplates themselves in duplicate and triplicate. This reproducibility ($p = 0.004$) makes the CLPP method statistically reliable for monitoring (Mulder 2006). In comparison to the compiled SSEO field data sets, the latter result seems surprising, but it is a strong indication for a much higher suitability of CLPP for **acute** stress (like the aforementioned study on short-term stress-induced changes in the rhizosphere due to the Cry1Ab protein from straw exudates) than for **chronic** stress.

26.4 Implication and possible use

The compilation of functional response data has thus improved the general insights into the design and outcomes of CLPP studies, so that they can be used to track functional ecological responses in the field. The above results do *not* imply that other possibilities to track chronic field effects of contaminants, like Pollution Induced Community Tolerance (PICT) measurements, may be seen as obsolete. We can conclude that the technique of metabolic fingerprinting by CLPPs enables the detection of *rapid* shifts in the functional diversity of the microbial community, as in the laboratory study with transgenic maize (Mulder et al. 2006), and the detection of *chronic* shifts, assuming sufficient large gradients and enough statistical power in the analysis (Boivin 2005). We have to conclude that the same method seems suitable for the detection of physiological shifts after an adaptive radiation (long-term stress), such as in the case of the Demmerikse polder, but not in the Biesbosch and ADW floodplains.

27 Spatial aspects in ecotoxicology: heavy metal accumulation risks in diffusely and moderately polluted floodplains

By: S. Wijnhoven, G. van der Velde, R.S.E.W. Leuven, H.J.P. Eijsackers & A.J.M. Smits

27.1 Problem definition

Ecotoxicological risk assessments and floodplain soil management generally focus on the regularly flooded parts, since metals are largely supplied during floods. Also, priority judgement is based on total soil concentrations with the binding capacity of the substrate only partly taken into account. However, floodplains are characterized by recurrent flooding and erosion, sedimentation and succession cycles, resulting in heterogeneous landscapes and dynamic interactions between aquatic and terrestrial systems. For partly flood-tolerant and flood-intolerant species, the landscape is therefore a changing mosaic of suitable and unsuitable biotopes, with elevated areas to survive floods and a certain connectivity. These species show mortality, population development and floodplain recolonization cycles related to flooding events. Among these species are small mammals which form important links in floodplain food webs. It has been shown that floods cause a large reduction of the small mammal populations, after which survivors are concentrated in non-flooded areas.

Recolonization of the formerly flooded areas is found to be a slow process due to a gradual population growth and a poor connectivity of the landscape. In embanked Rhine floodplains, the flooding frequency is generally about once a year. This leads to the presence of about half of the small mammal populations on and near (within 30 metres) the non-flooded areas, although these represent generally far less than 50% of the total floodplain area (Wijnhoven et al. 2005; Wijnhoven et al. 2006b). Since predators forage where prey is available, substantial parts of the vertebrate populations (e.g. small mammals and their predators) can be found in the non-flooded areas. We investigated whether exposure risks for vertebrates to metals are indeed highest in the regularly flooded parts.

27.2 Approach

The research was conducted in the Afferdensche en Deestsche Waarden floodplains (a focal area of the NWO-SSEO programme), which are characteristic of diffusively and moderately polluted floodplains in the Rhine delta. Two major exposure routes to metals for food webs, and small mammals in particular, are via vegetation and earthworms. To compare the metal concentrations between non-flooded and regularly flooded areas in floodplains, soil cores were taken from the top 10 cm soil layer at 58 sampling sites with various types of vegetation, soil and land use. Besides analysing soil samples for total and $CaCl_2$-extractable concentrations, at 27 sites (15 non-flooded and 12 regularly flooded sites), grass shoots (mixed samples of all grasses) and earthworms (mixed samples of all Lumbricidae) were also collected for metal analyses. All samples were analysed for metal content using Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES). Additional details can be found in Wijnhoven et al. (2006a).

27.3 Main results

Although significantly higher metal concentrations were found, as expected, in the regularly flooded areas, the risks of metal accumulation were not substantially lower in the non-flooded areas on basis of $CaCl_2$-extractable concentrations in the soil and concentrations in vegetation and earthworms (Figure 32).

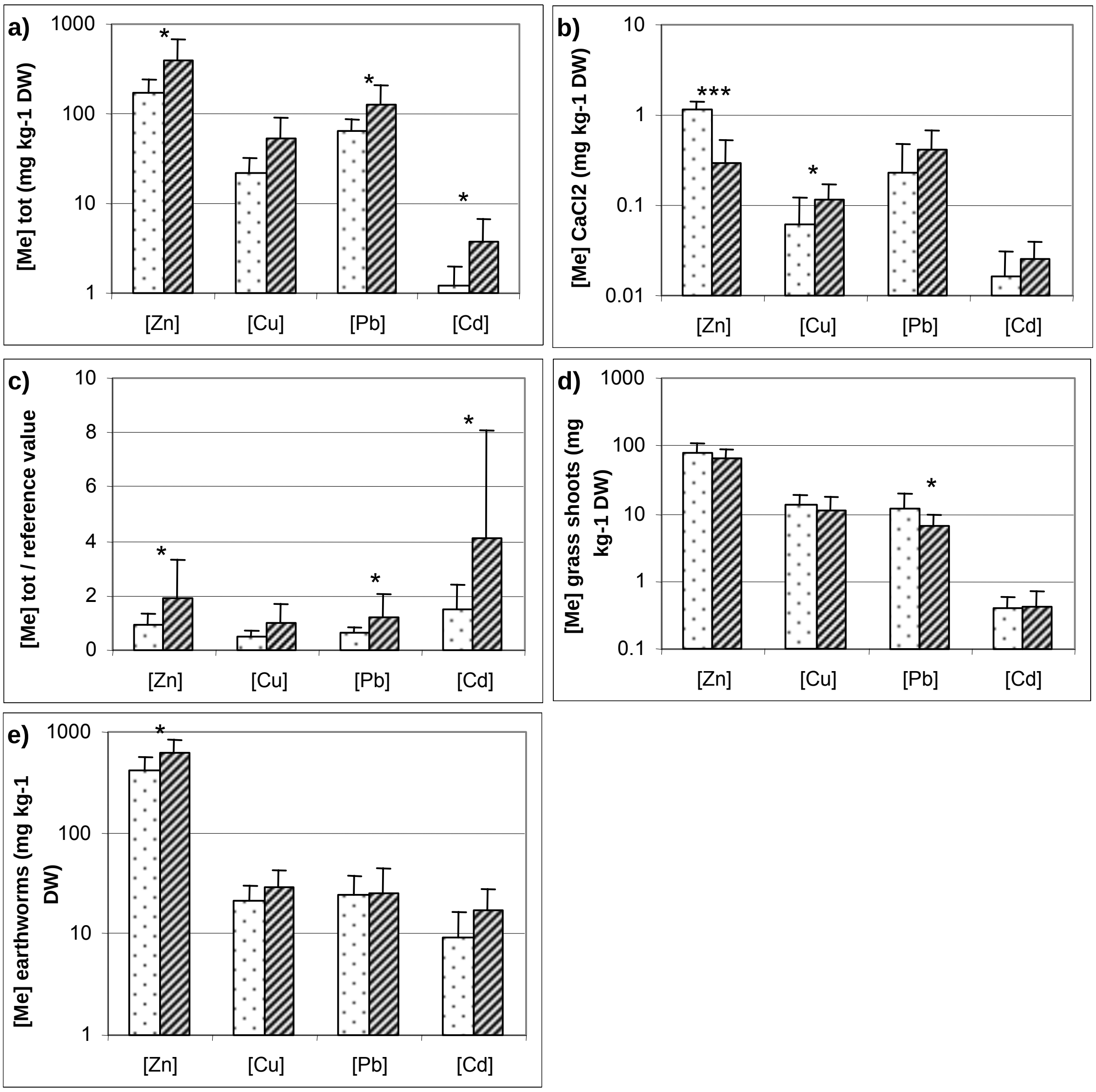

Figure 32. Graphs showing differences in metal concentrations (Zn, Cu, Pb and Cd) between non-flooded (light bars) and regularly flooded (dark bars) sampling sites. Metal concentrations are shown as (a) total metal concentrations (mg/kg DW); (b) $CaCl_2$-extractable metal concentrations (mg/kg DW); (c) total metal concentrations divided by the reference value corrected for organic matter and clay/silt content; (d) metal concentrations in grass roots (mg/kg DW); (e) metal concentrations in earthworms including their digestive tract contents (mg/kg DW). Significant differences are indicated by asterisks (*: P<0.05; ***: P<0.001), with n=15 for non-flooded sampling sites (except for (d) where n=13) and n=12 for regularly flooded sampling sites.

As regards soil parameters, the pH was significantly lower in the non-flooded areas than in the regularly flooded areas, which could partly explain differences in availability of metals. Differences between the investigated metals and exposure routes via vegetation and earthworms were observed; there were higher risks of lead accumulation in herbivores in non-flooded areas and higher risks of zinc accumulation in regularly flooded areas in species feeding on earthworms. But in general, the accumulation risks between non-flooded and regularly flooded areas were similar.

27.4 Implications and possible use

Extractable concentrations could be important for determining the risk of accumulation for certain soil organisms and their predators, which might include small mammal species that partially, locally or temporally feed on these soil organisms. Significantly higher risks of accumulation for small mammals in regularly flooded areas are expected only when soil ingestion is an important route of exposure, which remains an uncertain component in risk assessment. In another study, we measured metal concentrations in specimens of six small mammal species trapped at the same sites as investigated in this study. No relationship was found between metal concentrations in any the species and whether they were trapped in regularly flooded or non-flooded areas, with an exception for Zn in the wood mouse (*Apodemus sylvaticus*); this metal appeared to be significantly higher in the specimens trapped in non-flooded areas (Wijnhoven et al. 2007). Both studies showed that for ecotoxicological risk assessment, the total and potential available metal concentrations in soils and the concentrations in biota should be analysed. Taking major metal exposure routes for food webs and species distributions in floodplains into account, non-flooded areas of moderately polluted floodplains cannot be neglected in metal accumulation studies and soil management.

We concluded that the accumulation risks of metals, not only for in small mammals and their predators but also for other species, are determined by the status of the exposure sites. This is only a fraction of the total floodplain area. The sites at which exposure takes place are, of course, not necessarily the sites with high contaminant levels, but are those sites with suitable habitats, especially in and near non-flooded areas, or with a good connectivity with those areas. Floodplain soil management should focus on those areas and should especially prevent point sources of contamination there.

Section 4. Additional exposure and effect studies that broaden the scope

The following two chapters broaden the scope of the SSEO programme by looking at two monitoring databases. These databases were compiled from sample data collected in areas outside the three study areas, and were added mainly to avoid the final conclusions of SSEO being based on three areas with a possible bias towards sensitivity or insensitivity of ecosystem responses, while neglecting other types of areas. Furthermore, they were selected because of ongoing research by some of the research groups involved in the current project with a similar target as the SSEO studies. This section of the report is *not* a scientific review of all papers published under the auspices of the SSEO programme. As described in the project proposal (Posthuma et al. 2001), additional studies would be considered for various reasons.

28 Toxic effects, multiple stress and butterfly abundance trends in a nature area in the Netherlands

By: C. Mulder, D. de Zwart & L. Posthuma

28.1 Problem definition

Monitoring databases offer an interesting extension of the SSEO data sets, as they may give additional insights into the meaning of the field effects on biota of low-level exposure to diffuse mixtures of toxicants. In this study, we focused on a remote nature area in the province of Drente in the Netherlands, for which a vast amount of monitoring data is available. The study focused on population trends in butterflies, and analyzed whether observed declines can be related to local, diffuse exposure to toxicant mixtures. Attention was focused especially on the indirect effects on butterflies, mediated by the effects of toxicant exposure on host plants.

Plant reproductive structures inducing dispersal by animals (insect seduction) comprise the following strategies (Mulder et al., 2005a):
- ✓ psychophily (butterfly-related vascular plants),
- ✓ phalaenophily (moth-related vascular plants), and
- ✓ insect pollination due to other Endopterygota-like wasps, bees, ants, flies and beetles (hymenophily, melittophily, myrmecophily, myophily and cantharophily).

The presence of toxic compounds in the plant environment can have direct impacts on plants, and indirectly on insect seduction. Since each plant taxon is specifically related to a kind of pollinator, the question is whether changes in the density of pollinating lepidopterans (i.e., butterflies and moths) reflect the different responses of their host-plants to environmental pollution. This question is addressed in this study.

28.2 Approach

The long-term monitoring of butterflies was performed weekly by the Dutch Butterfly Conservation along a specific open-canopy transect (No. 0355) across the following vegetation types: Erico-Sphagnetum magellanici, Ericetum tetralicis and Lycopodio-Rhynchosporetum, all in the nature reserve area at Dwingeloo (Drente, the Netherlands). Although the study area is regarded as protected area, where pesticides, eutrophication and desiccation are assumed to not affect the investigated landscape, the combination of a strongly negative trend in the abundance of butterflies and an alarming biodiversity decrease (Mulder et al., 2005a) points to a disturbed ecosystem.

As the actual toxic effects of toxic compounds on field biota result from the presence of mixtures of toxicants, it was investigated what the association was between the net predicted toxic pressure of pollutants and the butterfly abundance data collected in the monitoring programme. The toxic pressure of contaminants is hereby defined by the parameter msPAF, the acronym for multi-substance Potentially Affected Fraction of species (De Zwart and Posthuma 2006). We use the term chronic toxic pressure when the (ms)PAF is based on chronic ecotoxicity data obtained from laboratory tests ($msPAF_{NOEC}$), and the term acute toxic pressure ($msPAF_{EC50}$) when this is based on acute ecotoxicity test data. Both types of msPAF define a characteristic of the abiotic environment, implying that with increasing (ms)PAF, the environment is more toxic for the laboratory-tested species (with the implication that a fraction of species are probably experiencing chronic and acute adverse effects, respectively), and (by assumption) likely also the local species. Evidently, a given environmental

$$ms\text{-PAF} = 1 - \prod_{i}^{n}(1 - \text{PAF}_i)$$

concentration will induce a higher fraction of species exposed to levels above the NOEC as compared to the fraction for which the EC50 is exceeded, whereby validation studies suggest that acute toxic pressure is a predictor of species loss (see Chapter 29). To account for the cumulative effects of different compounds, the individual-compound toxic pressures (PAF_i) were cumulated using the concept of response addition of pollutants (see De Zwart and Posthuma, 2006 and Mulder et al., 2005a):

28.3 Main results

<u>Analysis of msPAF for the Netherlands.</u> In the Netherlands, the 5%-PAF_{NOEC} level is considered "maximum tolerable risk", and ~0.05 % PAF is taken to represent "negligible risk". Although PAFs for single substances may range from well below 0.05% up to more than 10%, it should be noted that precise knowledge of ambient concentrations is essential.

Figure 33 shows that the ambient toxic pressure on the vegetation occurring in eight landscape units (Geographic regions) in the Netherlands is remarkably different. Although little quantitative information exists about ambient toxic pressures, it appears that toxic pressures (PAF_{NOEC}) on the vegetation of Dutch soils may range from less than 0.1 % in clean environments to more than 50 % in bogs and mires.

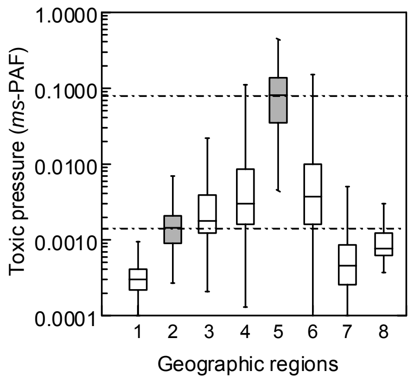

Figure 33. Chronic toxic pressure as function of different landscape units in the Netherlands (box plots of the study area are given in grey): Lower highlands (1), Northern Pleistocene sand (2), Southern Pleistocene sand (3), Fluctuating telmatic wetlands and riparian area (4), Permanent telmatic wetlands (5), Recovered marine clay (6), Dune belt (7) and wetlands and tidal areas (8).

<u>Analysis of msPAF for the study area.</u> The maximal chronic msPAF value for each species, defining the case where the occurrence of the species is not fully limited by toxicant exposure (thus, a measure of the ecological amplitude per species) has been plotted against the plant occurrence (expressed as amount of grid cells within the same landscape unit). This was done to test the actual relationship between the character states of the butterfly-related plants and the predicted plant sensitivity for heavy

metals. In Figure 34, the final configurations of the butterfly-related plants (black circles) and the complete species pool of the Netherlands (open circles) appears homogeneous, since both the regression lines narrow to an ideal case.

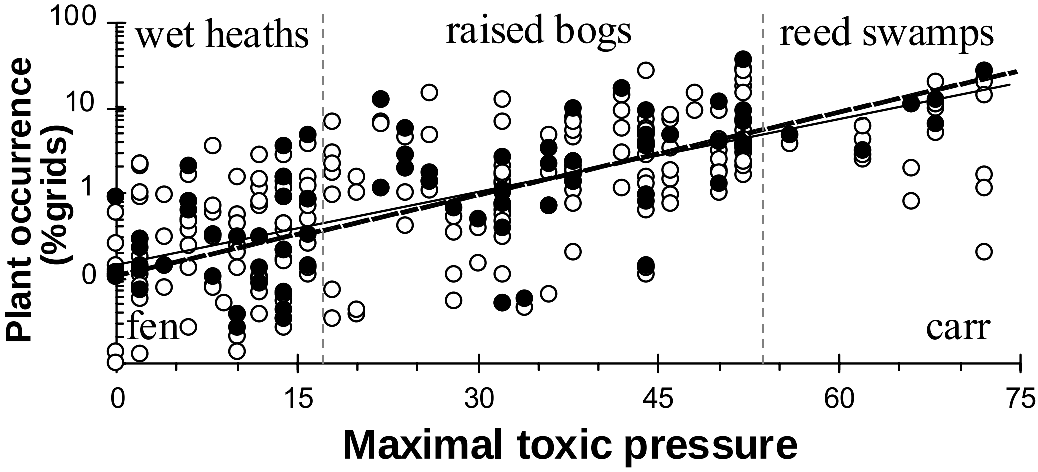

Figure 34. Maximal chronic toxic pressure (msPAF) in relation to the minerotrophic-ombrotrophic gradient of the investigated wetlands (gradient of increasingly rainwater-dominated systems). Each circle shows the highest multi-substance Potentially Affected Fraction (msPAF) possible for a certain species. Butterfly-related plants are plotted in black. Both regression lines are highly significant and show the same trend: the dotted, bold line represents butterfly-related plants (r^2 = 0.499, *n* = 65), the thin line the complete local species pool (r^2= 0.401, *n* = 209). Modified from Mulder and Breure (2006).

The scatter of the field occurrence of local plants in relation to the local value of the parameter msPAF shows an evident telmatic difference (Figure 34). Plants characterizing fens and wet heaths (like *Schoenus nigricans*, *Scirpus cespitosus*, *Narthecium ossifragatum*, *Oxycoccus palustris* and *Gentiana pneumonanthe*) have an occurrence related to very small potentially affected fractions (*ms*-PAF < 18 %; i.e. at higher msPAF, these species are not found in the field). In contrast, plant species related to raised bogs (like *Potentilla erecta*, *Eriophorum* spp. and *Molinia caerulea*) are biased in the middle zone (maximal *ms*-PAF = 36, 42, and 52 %, respectively; i.e. at moderate msPAF, these species do occur), whereas forested birch carr or reed swamps (characterized by *Typha angustifolia* and *Phragmites australis*) show a centroid in the upper right corner of Figure 33 (66 and 68 %; i.e. these species tolerate the higher exposure levels indicated by the higher msPAF). To a certain extent, the *x*-axis of this figure follows a gradient from open landscapes to closed canopies. It is precisely the host plants biased in the middle of this Figure that provide the nectar and pollen for mire-specialists like *Boloria aquilonaris*[14] (30 times fewer adults in six years) and *Vacciniina optilete*[15] (ten times fewer adults). In view of the high pressure of pollutants on these specific host plants (36 < msPAF < 52 %), the disappearance of such lepidopteran mire-specialists is not surprising at all, even in a protected area.

Pollination strategies can be easily clustered in relationship to the phenological character states of the observed plants; the occurrence of different pollination strategies (expressed as the number of species and as relative percentages) is given in Figure 34. According to this clustering, a rough ecological risk assessment shows a remarkable difference in the average metal pollution tolerance of vascular plants belonging to a given pollination group (Mulder et al., 2005a). Actually, nectar-plants for butterflies

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[14] In Dutch: Veenbesparelmoervlinder

[15] In Dutch: Veenbesblauwtje

(psychophily subset) are the only ones showing a lower resilience than the complete species pool (71 %), whereas nectar plants for moths (phalaenophily subset) are the most pollution tolerant (131 %). This can be easily related to the character states of the plant hosts of Lepidoptera, as 61 % of the butterflies and 66 % of the moths are strictly related to early spring-green plants. The key role within the species pool of the investigated wetland is played by hemicryptophytic graminoids (buds at ground level), which are regarded as a main target character state in most Dutch metal-polluted soils (Mulder and Breure, 2003).

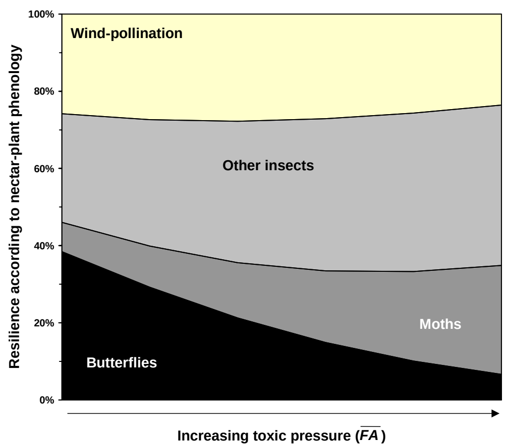

Figure 35. Relative tolerance of the 253 plant species occurring in Dwingeloo clustered according to their pollination strategy (Mulder et al., 2005a). The area under each curve is based on the environmental resilience (vigour) of a group of plant reproductive structures (insect seduction and phenology). The chronic msPAF (fraction of host plants probably affected beyond their NOEC) is scaled from 0 (left x-axis) to 100% (right x-axis).

28.4 Implication and possible use

This case study, executed as an extension of the validation/integration studies of the three SSEO study areas, showed that low-level contamination may lead to a sensitive cascade effect on lepidopteran field populations, related to the field sensitivity of host plant species to elevated mixture exposures. The direct responses of the plants to mixture exposure levels (quantified by msPAF) were determined under field conditions, and show up as the limitation of occurrence of a species when local toxic pressures exceed the apparent maximum value that can be tolerated by the species. Such a phenomenon (down-going trends for sensitive species being associated with up-going trends for opportunist, less sensitive species, was also reported in Chapter 29).

Together with other phenological character states in a given plant species, like the location of perennating buds, leaf seasonality and longevity, pollen production is crucial for microherbivores. To our knowledge, pollen itself has been almost neglected in plant-pollination interactions, although pollen concentration values are a reliable proxy for plant fitness in radio-traced palynological studies. It has been shown in fact that these pollen concentration values in later-stage heath communities can easily fluctuate by a factor of 2 to 3, whereas these simultaneous fluctuations are real changes in regional deposition values (Mulder and Janssen, 1999). When dealing with old *Calluna* stands at a stage of death and disintegration, the pollen fashion is not continuous and even in wet heath stands in earlier phases of development, the pollen concentration of most herbs is astonishingly low. Hence, fluctuating pollen production may have consequences for different insect species. Such intriguing relationships with air-pollution need investigation, since cumulative toxic effects on the local species pool require ecotoxicological assessments.

29 Analysing data from monitoring networks: the role of toxic compounds and other stressors in shaping natural assemblages of fish species

By: L. Posthuma, C. Mulder & D. de Zwart

29.1 Problem definition

Like all species assemblages in the field, fish assemblages in Ohio surface waters are known to be impacted by a broad variety of stress factors. Stressors include physical disturbance (e.g., canalization), water-chemistry change (e.g., altered pH), waste-water treatment effluents, and toxic compound mixtures. Various studies have identified the magnitudes of local impacts. However, it has only been possible to apply expert judgement to identify the causes of local impacts. This is a problem for river managers when they want to reduce local impacts by reducing the influence of stress. The manager is confronted with the question to identify which of the stressors locally affect the local fish assemblages the most. Knowing the impact is, however, insufficient to identify the causes. A causal analysis is needed for effective and informed decision making. When local river stretches can be under stress from toxicant mixtures at low concentrations, expert judgement usually falls short. It is highly unlikely that expert judgement is sufficient to uncover a role of such diffuse mixtures. Such judgements often only work for accidental spills in the river, where concentrations are high, and suspected causes are easy to identify (e.g., a discharge point). In this paper, we tried to identify and quantify local impacts, by ecological and ecotoxicological modelling, and to quantify local toxic pressures (see also previous chapters, e.g. Chapters 18 and 28). Our approach consisted of merging the scientific approaches of ecology with those of ecotoxicology and gradient statistics. Our aim was to identify and quantify local impacts, assign their probable causes, and clearly present both results for river management. The study is described in detail in De Zwart et al. (2006) and Posthuma et al. (2006). A similar study has been successfully conducted on monitoring data for England and Wales (unpublished data).

29.2 Approach

29.2.1 Identifying impacts and assigning probable causes

A three-step approach was followed to identify impacts and assign probable causes of impacts, as described in De Zwart et al. (2006):

- The first step concerned the quantification of mixture risks in Ohio rivers on a relative scale, the toxic pressure scale. This re-scaling of concentrations on an ecotoxicological scale allows for the quantification of the multi-substance Potentially Affected Fraction of species per site (see Chapter 28 for details). To diagnose the possible role of toxicant mixtures at low concentrations at different locations, the available monitoring database of stressors and fish species abundances was analysed. By means of ecotoxicological modelling (using the msPAF-approach), measured toxicant concentrations in the river systems were re-calculated into the locally multi-substance Potentially Affected Fraction (msPAF), making use of the SSD approach and established principles from mixture toxicology (De Zwart and Posthuma 2006). Although the separate compounds could not be associated to impacts due to statistical lack of power (each compound added reduces statistical diagnosis power), the msPAF appeared to show a clear signal across Ohio; the model of SSD, in combination with exposure and mixture assessment rules, generated for each sampling site a singular proxy parameter for toxic stress of mixtures, and this parameter correlated with fish species abundance for many individual species.

- The second step was the quantification of impact from field observations. The impact on fish assemblages was quantified for 695 sites, considering 100 different fish species, using an ecological model, RIVPACS, addressing the presence of absence of fish species at the sampling sites. The ecological model is often used to quantify impact, but it is not possible to assign probable causes. Hence, fish abundance data (the raw field data, see Figure 7) were translated by ecological modelling into local measures of effect (i.e., a quantitative measure of local impact).
- The third step combined the toxic pressure data, the RIVPACS-results and statistical gradient analyses in a method that attributes impacts (local species loss) to its probable causes. This resulted in the Effect-and-Probable Cause (EPC) pie diagrams (De Zwart et al. 2006) in which the pie size represents the local impact (measure of effect, box 3 in Figure 7), and the slice size the probable cause. Figure 36 gives an overview of an EPC pie diagram averaged over the whole monitoring data set. On average, the local toxic pressure contributed for approximately three percent to the explained variance in species loss. Note, however, that there is major variability across sites, with high acute toxic pressures occurring locally. Figure 37 presents EPCs for selected sites in four geographical areas with different dominant stressors.

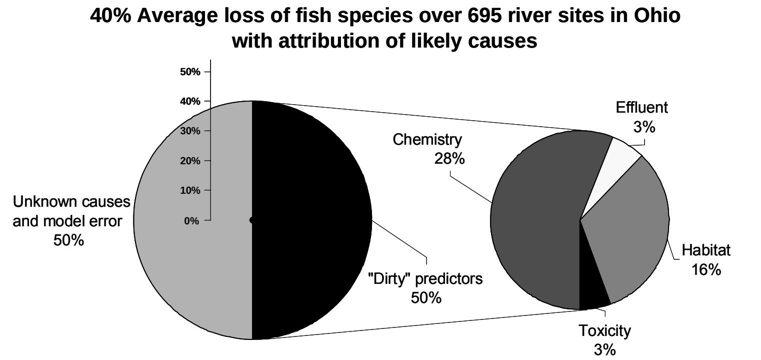

Figure 36. Attribution of changes in natural species assemblages of fish in Ohio surface waters to sets of site characteristics, averaged for the whole Ohio biomonitoring data set. Note that Figure 37 shows a wide variation of the relative relevance of stressors between sites and catchments.

29.2.2 Confirming the meaning of toxic pressure as predictor of impact

With the data treated as described, the validation of the toxic pressure model results against the local measures of effect became possible. To quantify the impact of mixtures alone in terms of species lost, the slice size that quantifies the relative role of mixtures in species loss was multiplied by the pie size (the total fraction of species lost). When this was done for all sites, this calculation resulted in the local, quantitative degree of impact assigned to mixture exposure, on a scale from 0 – 100%. This is the fraction of species lost attributable to mixture exposure. This is a scale with exactly the same type of interpretation as that of msPAF; both concern the fraction of species lost, but the EPC scale pertains to an *observed* loss, and the msPAF to a *predicted* loss. Hence, it became possible to study the relationship between msPAF and local loss of species (Posthuma and De Zwart 2006). The analysis is not a validation or confirmation in a strict sense; it provides an *empirical description* how msPAF relates to species loss. Scientists as well as regulators often ask for validation of the SSD-model. The reasons for this are that the SSD-model is a simple model that does not address ecological concepts in predicting species loss. It can thus be wrong. However, analyses like presented in this Chapter show that the use of the model can be helpful for various environmental problems.

29.3 Main results

29.3.1 Quantifying impact and stressor relevance per site

The complete set of analyses of the monitoring data resulted in the graphs in Figure 37. In this figure, it is shown that each site (out of 695 sites) has its own characteristic impact magnitude, and a characteristic combination of stressors apparently related to this impact. According to the model used (RIVPACS), each site was expected to have its own typical species assemblage in undisturbed conditions, and deviations from those expected conditions would show up as increased pie sizes. Furthermore, when this ecological impact quantification model is combined with the ecotoxicological results of SSD modelling (to quantify msPAF as local toxic pressure of mixtures at each of the 695 sites) and with multi-stress gradient analysis, the relative contributions of the different stressors to those impacts could be quantified. These relative contributions are shown as pie sizes. Together, the pies and slices presentation of local impact+causation defined the EPC pie diagrams.

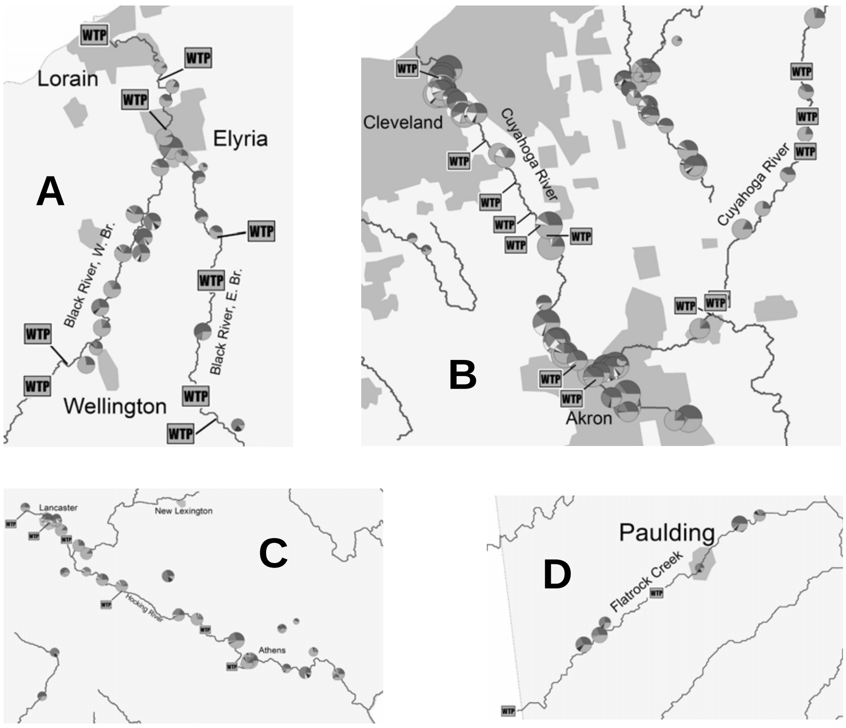

Figure 37. Geographically explicit presentation of local impacts of subgroups of related stressor variables on local fish species assemblages in Ohio rivers. The pie sizes represent the magnitudes of impact, i.e. the deviation of the local community from the community composition expected when no anthropogenic influences are in place. The slice sizes (grey shades) identify the major impact parameters, including the local toxic pressure (msPAF per site).

29.3.2 Confirming the toxic-pressure approach with field data

Further statistical analyses of the EPC results showed that the predicted msPAF (based on SSD modelling of *laboratory* toxicity data) had no direct (statistical) association to overall impact, i.e. when the impacts of all stressors are taken together (msPAF not correlated to pie size, n=695). However, a direct relationship between the predicted impact (acute toxic pressure) and the magnitude of impact that is attributed to the exposure to toxicant mixtures was found (P<0.001) (Figure 38). The analyses also showed large impact from other stressors (vertical spread of data points at a single predicted msPAF on the X-axis in the figure), which means that predicting msPAF solely is insufficient for obtaining a complete view of local impacts of stressor combinations. Such impacts may also occur due to other stressors such as (for the SSEO study areas) the inundation frequency at the floodplains field site (ADW) or the heterogeneity and patchy contamination in the Ronde Venen and the Biesbosch.

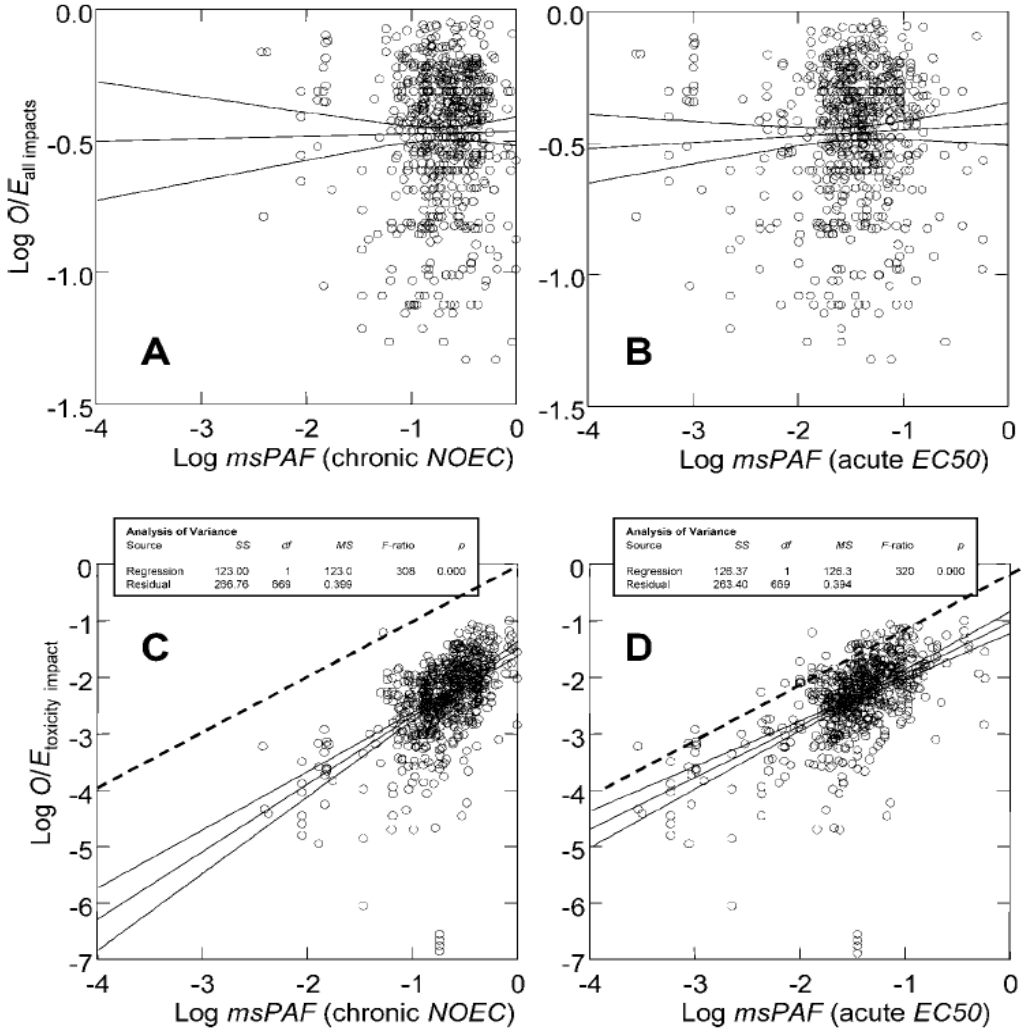

Figure 38. Relationship between predicted degree of impact (quantified by the msPAFNOEC, left) and the msPAFEC50 (right)) and the observed degree of impact (pie sizes, top) and when the impact is attributed to the effects of mixtures to the local communities (bottom, pie size multiplied by the slice sizes for toxic pressure).

In summary, the analyses of the large monitoring dataset of the Ohio rivers have led to the following key conclusions:

1. For each site, a unique combination of natural factors has been present, so that each site is characterized by a more or less unique, pre-disturbed “starting position”, defined by a specific species assemblage
2. For each site, a unique combination of stressors exists (levels and types), so that disturbed sites will differ by both degree and kind from other sites
3. Hence, a multiple-stress view is needed to analyse both the starting conditions and the stressed conditions.

29.4 Implications and possible use

Various validation studies have been conducted on the concept of SSDs. The majority of the studies made since the inventory and first use of SSDs focused on the question whether an SSD-based estimate for the HC5 relates to a limit concentration beyond which effects can be observed in field conditions. This appeared to be the case (see Chapter 15 for exceptions). Some studies looked into the relationship between SSDs based on laboratory toxicity data and those derived from the densities of different species along field gradients or under experimentally manipulated field conditions (Emans et al. 1993; Okkerman et al. 1993; Posthuma and Smit 1999; Posthuma et al. 1998; Smit et al. 2002; Van den Brink et al. 2002a; Versteeg et al. 1999). The present study is the first that addressed the SSD validation issue from the perspective of real multiple-stress field data.

The use of the methodology for analysing monitoring data can become important for regulatory use in the near future. Many biomonitoring efforts are currently being made, especially within the framework of some specific regulations. For example, the EU Water Framework Directive specifically focuses on good ecological status (GES), to be reached at some point in time in the near future – as an addition to the stressor-focused approach which requires that toxicant concentrations should be lower than criterion values. The same holds for EU-REACH, whereby regulatory agencies may need to consider the net probable impact caused by an array of chemical production facilities (e.g., in a river catchment). Monitoring schemes are currently designed to enable the consideration of GES. In case GES would not be reached, an integrative method like the EPC method could be used to identify not only the presence of impacts (no GES), but also its probable causes. With the latter, site managers would be enabled to take remedial measures, or define appropriate “next steps” in a risk assessment framework.

Section 5. General discussion and conclusions

This section reviews the main findings of the research activities that are reported in the previous sections. It specifically addresses the main targets of the research :

1. compiling the data of the researchers in the SSEO programme
2. validating models using the compiled results to investigate the degree of validity of selected models, and
3. initially integrating the programme results and disseminating the results by obtaining an overview of SSEO results and formulating options for applying the results of the programme in the regulatory context.

The results of the above activities are compared with other scientific evidence. Then the insights obtained are used to address regulatory issues. Although the research of SSEO did not focus on answering specific regulatory questions (such as site management for the three research areas), various remarks on this topic can be made. In this sense, the implications of the findings of SSEO for various regulatory fields (toxic compound regulation, pesticide regulation, handling already contaminated water, soil, and sediment volumes, and nature protection and species protection policies) are discussed. A summary of these discussions has been published in a Special Issue of the journal *Bodem* (Posthuma and Vijver 2006), in the *Dossier* section of the journal *Milieu* (Posthuma et al. 2006b) and in a Special Issue of *Science of the Total Environment* (Posthuma et al. Subm.).

30 Characteristics of the compiled data sets

By: L. Posthuma, M.G. Vijver & F. Kuenen

30.1 Background and motives

The Steering Committee and Programme Committee of the SSEO programme planned from the onset to integrate the research findings of all projects at the end of the programme (NWO 1999). A primary aim was to validate a suite of models currently used for regulating toxic compounds using the compiled data of SSEO. A second aim was, clearly, to integrate all programme results and translate the scientific findings into options for improving regulations. The present chapter reviews the results of the data compilation, the first step towards for integration and model validation. Data were compiled because:

1. Compiled data can result in statistically *more powerful* analyses; this yielded results that could not have been obtained by individual researchers working on separate data sets,
2. Compiled data can result in *additional* scientific analyses, like a food web analysis in addition to a food chain analysis, and a food chain analysis in addition to single-species exposure data.
3. Compiled data, in combination with data sources from outside the SSEO programme, are helpful in considering the applicability of SSEO findings to other study sites.

30.2 Results

The compilation of SSEO data resulted in three data sets, one for each SSEO research area. These data were made available for other researchers in the SSEO programme; the results are reported in the previous chapters. Analysis of these compiled data showed a huge variability in measurement endpoints.

First, the compounds that have received the most research attention and that are present in the substrates of the study areas are heavy metals (Zn, Cd, Cu, Pb) and to a lesser extent PAHs (Polycyclic Aromatic Hydrocarbons). In the freshwater tidal area, measurements showed the presence of elevated PCB concentrations.

Second, there is an array of parameters on exposure, mostly consisting of operationally defined measures of contamination, like total concentrations of local compounds, dissolved concentrations, $CaCl_2$-extractable concentrations, and exchangeable concentrations. This list is extended by body residue measurements in a variety of species. To allow for understanding exposure, an array of substrate characteristics has been measured, like substrate acidity (e.g., pH_{KCl}, pH_{H2O}). The data compilation was conceptually relatively easy for exposure-related data; this is because data related to external concentrations, which are sometimes distributed over different fractions, and data that relate to body residues, all have the “same currency”. All these data for a compound (e.g.: Cd) can be expressed in SI units, such as concentration in mol/g-units. All the variables can easily be harmonized and compiled into a single, larger data set for further studies. For example, the analyses of Veltkamp et al. in this report have shown that such compiled data are useful for exposure-model validation and for model improvement. By compiling exposure-type data, an integrated view of SSEO results of this kind can be obtained relatively easily.

Third, a large variability of parameters has been found in the SSEO data on biotic responses. This is logical, since the researchers focused on an array of effect-related endpoints, whereby, for example, the abundances of individual species in a sampling plot already resulted in a broad array of response parameters (an abundance variable per species). Other examples are the occurrence of Pollution-Induced Community Tolerance and the observations made for establishing this tolerance response in individual wells in multi-well plates. The results of PICT data can most often not be "translated" to a density change of earthworms across a gradient, or to nematode species diversity. Due to the variability of response parameters, it was more complicated to address the abovementioned integration and validation targets with effect-related data. Nevertheless, there may be possibilities for deriving novel aggregate parameters. For example, it might be possible to calculate a measure of biological diversity like the Shannon-Wiener index for each sampling spot in a sampled SSEO area, in addition to some data sets on species abundance data. However, this will require interpolation and modelling techniques to fill out "data gaps", for example to analyse response patterns in relation to exposure patterns. It is to be expected that such research will have limited success, since even regular monitoring efforts (designed to result in a meaningful and comprehensive compilation of data that are ready for impact and cause analysis, as in Chapters 28 and 29) are often relatively incomplete and inconsistent (see Section 4). As compared to planned monitoring, note that the SSEO data collection efforts were more limited (with respect to completeness and consistency) than efforts made for regular monitoring.

30.3 Conclusion

The data from many studies in the SSEO programme have been compiled and have been used for some successful additional data analyses. This extended output of the SSEO programme has been summarized in this report and in various underlying scientific publications on model improvement and validation. The expected added value of the combination of all data (more statistical analysis power for one study endpoint after combining data, and more study endpoints by combining separate endpoints) has largely been worked out in the present report. However, there were limitations encountered due to variability, mainly of the effect-oriented endpoints. Furthermore, added-value assessments might be made on the basis of the compiled data, but this requires various assumptions and interpolations.

31 Integrating scientific findings of the SSEO programme

By: L. Posthuma, M.G. Vijver & F. Kuenen

31.1 Contents of this chapter

This chapter makes a first attempt to integrate the scientific findings of the SSEO programme. It is based on the previous chapters with validation study examples (Section 2) and the study examples that were selected to represent the case studies of SSEO and other sources that were *not* related to validation of models (Sections 3 and 4).

A formal scientific review of the programme would require hypothesis-driven analyses of the findings in relation to all relevant literature, which was beyond the scope of this study.

This chapter addresses the occurrence of a large variability of responses, and tries to find the scientific explanations for the observed variability. After this analysis, attention is focused on the issue of model validation (in the next chapter).

31.2 General exposure and effect issues in cases of diffuse pollution

The SSEO programme has demonstrated that diffuse pollution can result in exposure of and effects on field organisms, ecosystem structure and functional aspects of ecosystems. Diffuse pollution was mainly studied in the three selected areas, but additional information from other studies in the Netherlands and elsewhere suggests that diffuse pollution is a frequently encountered condition in Dutch and other ecosystems.

Due to the long-term presence of compounds in soils and sediments, and associated processes like sorption to the matrix particles, it appears that the physicochemical availability for uptake (the "supply" offered by the environment) is often – but not always – lower than that usually present in laboratory test systems, which often assume "full availability" (See Chapters 8 - 12). This implies that the exposure ("biological availability") in field conditions is often lower than anticipated, and the effects under field conditions can likewise be limited (due to "toxicological availability"). The discrimination between these three aspects of availability studies was introduced by Dickson et al. (1994). Despite the often low availability in field soils and sediments, both an array of exposure levels and an array of effect types and magnitudes were established, ranging from functional effects on microbial communities (Chapter 25), to probable effects in populations of species higher in the food chain (Chapter 13). In some cases, the effects were latent (Chapter 24); they were visible only when additional stressors were applied, which in those cases were exhibited by different sensitivities of pre-exposed and reference species assemblages. In various cases, effects could only be distinguished from the responses to other stressors by approaches like population modelling and ecological modelling at the community level in combination with ecotoxicological modelling (Chapter 20), and by other diagnostic approaches with large monitoring data sets (Chapters 28 and 29). There were no signs of a clearly visible "synergism", i.e. a positive interference between contamination effects and other stressor infuences, so that the observed responses could be considered to be far higher than expected from simple response additive effects.

The problem of recognizing an array of exposure and effect types and magnitudes implies a problem for chemical regulation. Should chemical regulations be adapted, or should additional approaches be considered? This question has driven many of the efforts of the validation and integration project. Issues relevant to this question are also described in this section.

31.3 Variability of responses and a key conclusion of SSEO research

The major research problem of the SSEO programme, related to the regulatory problems with diffuse pollution, is the recognition of field effects in realistic ecosystems caused by exposure to diffuse pollution. There are two major questions:

- scientific question: "what is – or are – the ecological effects of diffuse pollution?", and
- regulatory question: "which implications should the effects of diffuse pollution have for various regulations?"

An overview of the published papers that are based on the SSEO programme, and on the Chapters of this report, suggests one universal scientific conclusion:

- *the field responses of biota that are chronically exposed to toxicant mixtures at low-to-moderate and sometimes high concentrations are highly variable. More specifically, at high exposure levels, effects may be absent or small (for example, concentrations exceeding the intervention value in soil), but at ambient concentrations that are considered rather safe. there may be visible effects.*

Some researchers did not find any effects on the organisms studied or found few effects at relatively high ambient concentrations, while others provided evidence that increasing exposure levels relate to an increasing change of biological integrity, both at the level of protected species (Chapter 28) and on the species composition of local communities (Chapters 22 and 29). In detailed studies on an array of measurement endpoints (effect types), many examples of typical, species-specific responses are provided in the research papers of the SSEO programme. In the SSEO-studies, the exposure was sometimes higher than the regulatory intervention value for various compounds (which has always been interpreted as indicative of the presence of serious effects). In the latter case, probably due to the size of the data sets, pollution-related changes in species and species assemblages could be filtered from the effects of other stressors at exposure levels that would imply "low to negligible risk". Further pattern analysis of the compiled responses is possible. Such analyses will most likely *not* show that the array of responses induced by diffuse pollution can be arranged or understood according to some simple laws. Understanding the responses requires insight into all issues on the pathway from cause to effect: the source – path – receptor model, a classical concept in toxicology and ecotoxicology. This is the conceptual basis for addressing possible answers in the remainder of this report.

The major scientific conclusion implies the presence of large regulatory problems, given that the regulatory criteria for "clean ecosystems" are frequently exceeded:

- *how should exceedances be handled in regulatory practice, regarding the nationa and internationall set of regulations on chemicals, compartments (water, soil and sediment) and protection of biota (species and ecosystems), and regarding site management in cases of existing diffuse pollution?*

In other words, various regulations address diffuse pollution, and the question is, how can they be linked to each other? Moreover, how can they applied in practice on existing contaminations, for which cleanup is not a realistic option? Note that there is less concern for regulatory problems with novel cases of pollution; for novel cases, regulations often prescribe immediate cleanup, which is a responsibility of the problem owner. Hence, in the remainder of this report, we will focus on existing diffuse contaminated problems.

31.4 Main causes of variability in responses

Before options for solutions of a problem can be proposed, the problem must be understood. The causes for the variability of responses of biota to diffuse pollution must be unravelled, and given the SSEO data and the additional literature, this can be done using the risk paradigm (Figure 8) as a working hypothesis.

Risk is defined by the probability and magnitude of effects. According to the risk assessment paradigm, this implies exposure assessment, sensitivity assessment and risk characterization. For the exposure assessment, the SSEO and additional literature data show that responses will strongly depend on true exposure under field conditions. The true exposure under field conditions is influenced strongly by the sorption of the compound to the local matrix (soil, sediment or water). During this process, sorption is strongly influenced by matrix characteristics. It is too simple to use the generic estimate of exposure that is often used in the process of generic risk assessments also for site-specific assessment. The degree of sorption of compounds to matrices differs strongly across sites (soils, sediments, water bodies), and thus a site-specific assessment of risks should show large differences between the effects of a single mixture (total concentrations) across sites.

For the effects assessment, the SSEO and additional literature data show that responses also strongly depend on the sensitivity of the exposed organisms. Some organisms are sensitive, others are insensitive. Sensitivity differences can not only occur between very distinct species, but also between taxonomically closely related groups or species. The concept of Species Sensitivity Distributions is based on this phenomenon. For this reason, the effects of a certain compound mixture at a diffusely contaminated site will depend on the exposed species.

In the context of sensitivity differences between species and species groups, it is noteworthy that the SSEO study areas may be characterized by a bias. The study areas are relatively dynamic, and are likely populated with species that can withstand general stress and environmental variability and unpredictability better than many other species (see working hypothesis formulated in Chapter 24). As a result of general stress-tolerance characteristics, the effects of one kind of stress (diffuse pollution) may be relatively limited in such areas. For this reason, several additional studies from outside the SSEO programme were added to address the issue of mixture effects on other groups and in more stable systems. Extension of field-impact studies to some more sensitive locations is generally recommended to avoid unjustified generalizations from highly dynamic systems to all systems.

Another bias may be that the studied compounds are mainly metals and PAHs, and that only some studies focused on specifically acting compounds (like insecticides). Diffuse contamination with specifically acting compounds may affect specific species groups (like insects), and this requires special attention in retrospective risk assessments (Jager et al. 2007; Posthuma et al. 2002a).
In conclusion, the large differences in apparent responses to diffuse pollution are related to three factors, which are in turn related to the *source – pathway – receptor* concept:

- differences in mixture composition and total concentrations
- differences in sorption of the compounds to the local matrix (causing differences in true exposure), and
- differences in sensitivity between exposed organisms.

Site-specific and often unique combinations of these three factors determine whether or not exposure takes place, and whether or not this results in observable adverse effects.
When this conclusion is interpreted in the context of generic environmental quality criteria, it becomes apparent that exceedance of generic quality criteria like the target value or maximum tolerable risk

(MTR) should be interpreted scientifically as a first sign that effects may occur – and not as precise predictor of the kind and degree of effects. In a regulatory sense, however, the interpretation of these criteria remains the same: exceedances are a signal to trigger regulatory action. Expected exceedances of the target value can induce actions like limitation of emissions, or non-acceptance of the marketing of a compound. Observed exceedances of the intervention value (in the current Dutch situation for polluted soils) trigger further research to consider the urgency of site remediation (VROM 2006a). In short: exceedances trigger action, either in the sense of management (first example) or of further research (second example). Both are effective instruments for environmental management – although exceedances *per se* just do not indicate with scientific precision the kinds and magnitudes of ecological effects. Options to solve this problem are provided in the next section.

31.5 Elaborating on source – pathway – receptor issues and response variability

31.5.1 Source- and pathway related issues

The occurrence of ecological effects of diffuse contamination is logically related to the occurrence of toxic compounds in the environment. This is the ultimate source of toxic effects, although the ultimate effects are always modified by other stressors and other factors (like pathways and sensitivity). SSEO research has shown that the source of effects is highly variable across the research areas (Chapters 3 - 5). There are gradients, as planned according to the design of the programme, although various gradients were not represented by a simple transect (continuous low → high exposure increase), but are dependent on multiple samples (together being a set gradually different samples). Gradients were found for various compounds, resulting in mixture of compositions that can be considered unique for every sampling site. On this basis, the research has shown that the pathways vary between sites and between organisms. In various instances, the exposure level is strongly influenced by local factors, like the exposure to organic contaminants in relation to Black Carbon (see Chapter 10). Factors like Black Carbon for organics, and pH for metals, strongly influence sorption of chemicals to the substrate, and this implies limited exposure, even when total concentrations of toxic compounds are high.

In the SSEO programme, various studies focused on aspects of the pathway. Many of these studies succeeded in linking body residue levels of organisms collected in the field (or in bioassays with field collected material) to the sources in the environment, by considering modifications of exposure caused along the pathway. Despite the fact that compound concentrations have exceeded the intervention value at various sampling locations, uptake in organisms appeared to be limited in various studies (Chapters 8 - 10), suggesting a role of the matrix in reducing the availability for uptake, by means of increased sorption. This process has been named “ageing”. It also appeared that the availability for uptake depends on local conditions, and that the biological activity of burrowing animals could change compound availability. In various studies, for example Chapter 10 on organics and Chapter 11 on metals, improved exposure models could be derived. These models can be applied to other sites, to predict expected levels of true exposure under ambient conditions. This is important for planning and implementation measures to reduce or mitigate the risks of existing contamination, given the fact that large areas with diffuse pollution cannot usually be remediated.

31.5.2 Effect-related issues

Many SSEO studies focused on the presence and magnitude of effects of diffuse contamination. It was suggested that effects may be *absent* when the exposure is limited, which occurs when the total concentrations are low, or when pathway characteristics limit exposure. When there is exposure, *effects of exposure may be smaller or larger*, depending on the true level of exposure and the sensitivity of the exposed group or the studied measure of effects.

The more a measure of effect is associated to the cellular interaction between toxicant molecules and receptor sites, the easier it will be to discern responses from natural variability, assuming that sensitive measurement techniques are available for all levels of biological organization. Biomarkers are conceptually a more sensitive measure of effect than parameters like biological diversity, since they respond to molecular or cellular interactions between the compound and the organism. Only when this kind of damage occurs frequently will it affect individual and population viability, and only thereafter will community-level parameters be affected. This expected pattern is illustrated in Figure 39, and this expectation should be taken into account when interpreting the array of responses found in the SSEO programme (of which an overview is presented in this report). The response types varied from biochemical and physiological response parameters measured in individuals to population-level and community-level effects. In the latter case, sensitive species disappeared, and apparently opportunistic species occupied the empty niches, up to the exposure level at which these species also begin disappearing.

It should be noted that all these responses (and the apparent absence of responses in various cases) were observed in the three study areas, for which it is known that the pollution has a long history. Like the process of “ageing”, which reduces availability, this may imply that the studied organisms have been genetically adapted to the local conditions, which in turn implies that they are probably less sensitive than conspecifics from reference areas. A specific example of this is the demonstration of PICT, Pollution-Induced Community Tolerance. While populations of species may respond to contamination by genetic adaptation and physiological acclimation (Klerks and Weis 1987; Macnair 1993; Posthuma and Van Straalen 1993) – both being an indicator of pollutant stress – community-level responses like PICT indicate loss of sensitive species and adaptation of others (Blanck et al. 1988).

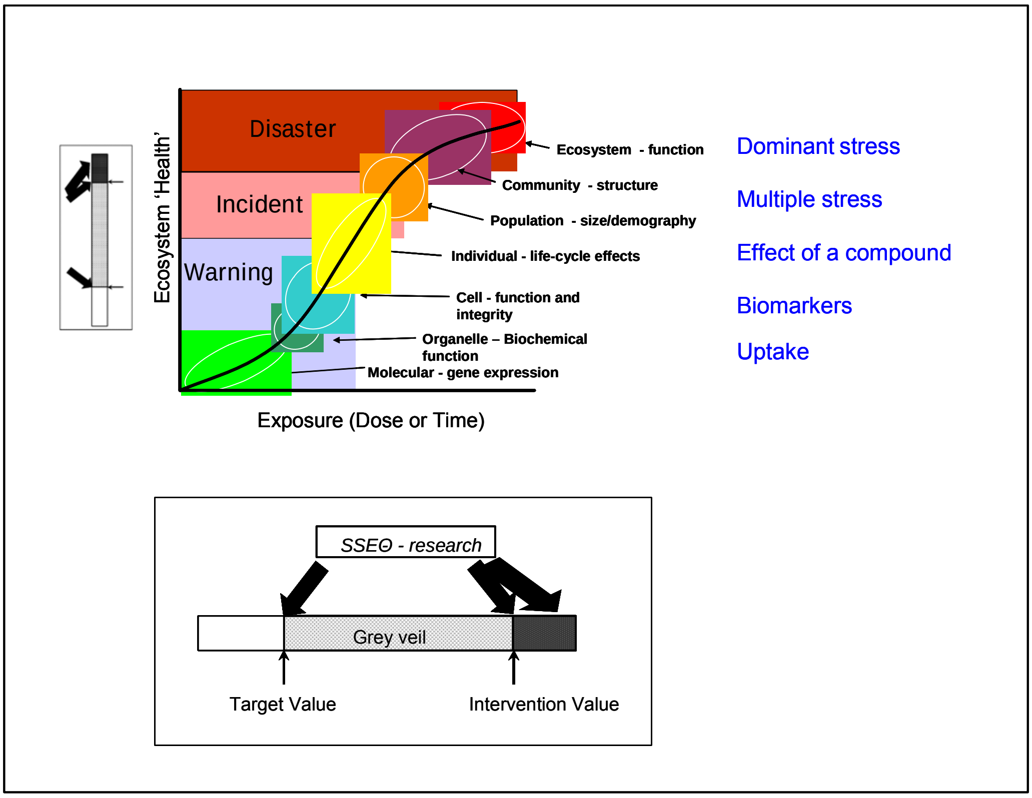

Figure 39. The relationship between the problem definition of the SSEO programme (below, relationship with generic quality criteria) and the array of possible measures of effects, ranging from molecular interactions between toxicant molecules within the organisms" tissues, effects on cell and organ function, population-level effects in separate species to massive disaster at the community level. Except for massive disaster, the effect signal is more difficult to establish when going from lower to higher levels of biological organization, while the "ecological relevance" (relationship to protection endpoints) increases. Amended from the concept presented by Spurgeon.

In addition to direct effects, *indirect effects* may also occur. For example, when some species are sensitive to the exposure (and exhibit decreased abundance or disappear), others can make use of the empty niche. Such indirect effects have been observed in the eco-epidemiological study of fish species in Ohio (Chapter 29), where it appeared that some species show a positive association to the toxic-pressure variable (for graphs and correlations, see original research papers). This implied that a parameter like "biodiversity" can remain apparently unaffected for an array of exposure levels, while in the meantime sensitive species exhibited reduced abundances and opportunistic species increased abundances (De Zwart et al. 2006). This was found earlier by Smit et al. (2002), and is also discussed in Chapter 24. A striking example of an indirect influence is the abundance change of butterfly species as a consequence of a sensitive cascade of effects (Chapter 28). Next, *effects may be latent*, and may only be uncovered experimentally. This apples for example to the presence of latent effects in nematode communities (see Chapter 24), and for the concept of PICT (Chapter 25). In the former case, there were various differences between exposed and non-exposed nematode communities, but the major difference was the alteration of response to a secondary stressor (heat shock) in comparison to non-exposed nematode communities. In the latter case, the method to identify PICT makes use of a secondary-stress exposure to find evidence for pollution-induced inherited changes in tolerance. Finally, *other stressors* are also relevant in shaping assemblages of species, making it difficult to establish the relationship between effects and probable causes. Nonetheless, based on comprehensive measurements for the toxicant-related source – pathway – receptor variables as well as for other variables, various studies resulted in quantifying the relative importance of diffuse pollution to toxicant mixtures as compared to the role of other stressors. See Chapter 18 for various areas, Chapter 28 for butterflies and Chapter 29 for fish in surface waters.

Despite factors that could *limit* the magnitude of effects, there may be one factor that could increase effects: synergism. Although it has been established that many stress factors shape local species assemblages, the SSEO programme has not provided clear evidence for the presence of synergistic effects of diffuse pollution in combination with other stressors. In general, multiple stresses acting together induce larger responses (cumulation of responses), as discussed in Chapters 18, 20 and 29, but not significantly *more* than expected from predictions for the effect cumulation of two or more independent effects that occur simultaneously.

For the three study sites, it was implicitly assumed that all researchers would be able to find impacts, since quality criteria were being exceeded. That impacts could be large, but also small or hidden, relates to all factors mentioned above (field factors), but also to the way quality criteria are derived. As described by e.g. Sijm et al. (2002), a legally adopted quality criterion is often the most stringent limit that is obtained from both a human-oriented and an ecology-oriented risk assessment, and this stringent value is then often adopted as legal quality criterion. Hence, in case of exceedance of a criterion, this may imply either human or ecological risks, or both. Moreover, even when a legal criterion is derived from an ecotoxicological risk limit, exceedances do not usually serve as a guide to impact magnitude. In various cases, the criterion has been derived by a applying a safety factor, in relation to the preventive context. The lowest known laboratory-derived NOEC is, for example, divided by such a factor (say 10, or 100). When the final criterion is 10, as an example, and derived using a Safety Factor of 10, can we know what it means if the concentration is 20? We can't. We can only say that 20 is five times lower than the lowest NOEC, which was 100. Due to this type of effect, the use of risk quotients (defined as the ratio of local concentration divided by the criterion value) also may not provide a clear indication of ecological impact. Solomon and Takacs (2002) summarize why risk quotients are relatively unspecific.

From all the abovementioned perspectives, it can be understood that various studies did not show adverse effects of diffuse pollution, and that observed responses were highly variable. A general conclusion on finding effects when they are there is that it is more likely to find such effects when:

- the exposure is higher and concerns more compounds (both total concentrations, and when there are limited effects on exposure caused by the exposure pathway, like sorption)
- the measure of effect is sensitive
- the influences of confounding factors are limited or taken into account appropriately
- care is taken to look at direct and indirect effects and at latent effects
- there is a dataset of sufficient size, containing sufficient information on the gradients of all possible causal factors in addition to the pollution gradient

Finding effects and attributing them to diffuse pollution requires a methodologically appropriate study design with sufficient data (Chapters 21, 18, 28, and 29) and with sufficient attention for all other stressors. In every case, observed field phenomena are the net effect of the local history and all stressor variables. When multiple stress observations are made, the net effect of mixtures can (in a site-dependent way) be lower or higher than those of the other stressors (Chapters 18, 25, 28, 29). When studying two sites (e.g., one contaminated field site and a control site), clear statistically significant "effect" differences can be found. It is, however, methodologically incorrect to assign those differences to any hypothesized factor that differs between the cases (such as diffuse pollution), because the sites will differ anyway (due to other causes).

Failure to demonstrate responses, as in some SSEO studies, is not necessarily evidence for the absence of responses. Responses may be present or latent, they may occur at lower levels of biological organization than observed, etc. And only appropriate and powerful designs, adapted to the local signal-to-noise ratio, will enable proving the presence of mixture effects. To prove the absence of responses – or better, to provide evidence that responses are limited – requires a power analysis of the study that was undertaken. When the study design is low in power, any conclusion that there are no responses is weak.

As a result of conceptual reasoning and based on the data from the studies on biomonitoring data sets in the Netherlands (invertebrates in river systems, butterflies in Drente) and elsewhere (fish in Ohio rivers), the following conclusion can be drawn about the differences in effect levels:

- There is evidence for a general, continuous increase of local risks of diffuse pollution when ambient concentrations increase (modified by factors mentioned earlier); in the field, there is no discontinuity in responses like one that could be associated with risk limits as derived from generic risk assessments (such as "no responses below the target value, and presence of responses above it").

31.6 Practical implications of variability in exposure and responses

In the previous sections, it was shown that local responses of biota to diffuse pollution are determined by the combination of the compounds, their concentration, the matrix and the sensitivity of the exposed biota, evidently modulated by temporal and spatial factors. Larger areas and longer exposure are generally considered to be more risky than short-term, small area exposure. This finding has a general consequence for regulation of handling diffuse pollution. It implies that a clear distinction should be made between preventive, compound-oriented regulations (like the recently adopted EU-REACH), and the assessment of site-specific risks. Although the same risk paradigm (problem formulation + exposure assessment + effects assessment + risk characterization) is used (see Figure 8), the dissimilarity between both approaches is the amount of site-specific parameters that can be used to assess risks in the latter case.

As a solution, the concept of a tiered system for risk assessment can be adopted (Figure 40), whereby the lowest tier consists of an evaluation using generic quality criteria. This assessment is influenced by ecotoxicological approaches, by a generic exposure scenario and a risk limit derived from laboratory toxicity tests. In case these criteria are not exceeded, there is no regulatory trigger to consider further action, or to do more research (Figure 41). In case of exceedance of the generic criterion, there is doubt as to which types and levels of effects are to be expected, and whether there is a real need for risk management. In such cases, based on the same risk paradigm, more site-specific information can be used to make a refined site-specific assessment. This may involve site data that are not only used in the classical ecotoxicological exposure and effects assessments, but also ecological site data. Further refinements can be made, with increased focus on specific site and ecosystem characteristics, local management targets and options for improving ecological quality. Optionally, scenarios can be made to compare risk management options. Further details on tiered approaches will be published elsewhere soon (Posthuma et al. in press). The tiered system can be used as a solution for the problem of diffuse pollution, in view of the regulatory application of such tiered assessments in other fields, like the regulation of plant protection products in the EU, (European Union 1991) and the assessment of remediation urgency in the Netherlands (VROM 2006a), based on the earlier Sanitation Urgency System (as second tier for cases of soil pollution where the intervention value is exceeded (VROM and Van Hall Instituut 2000)).

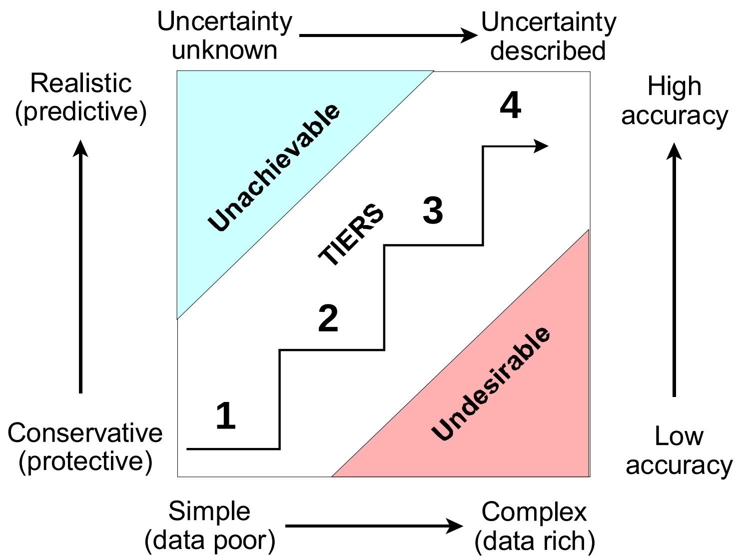

Figure 40. Key characteristics of a tiered system in risk assessment processes, after Solomon et al. (Solomon In press).

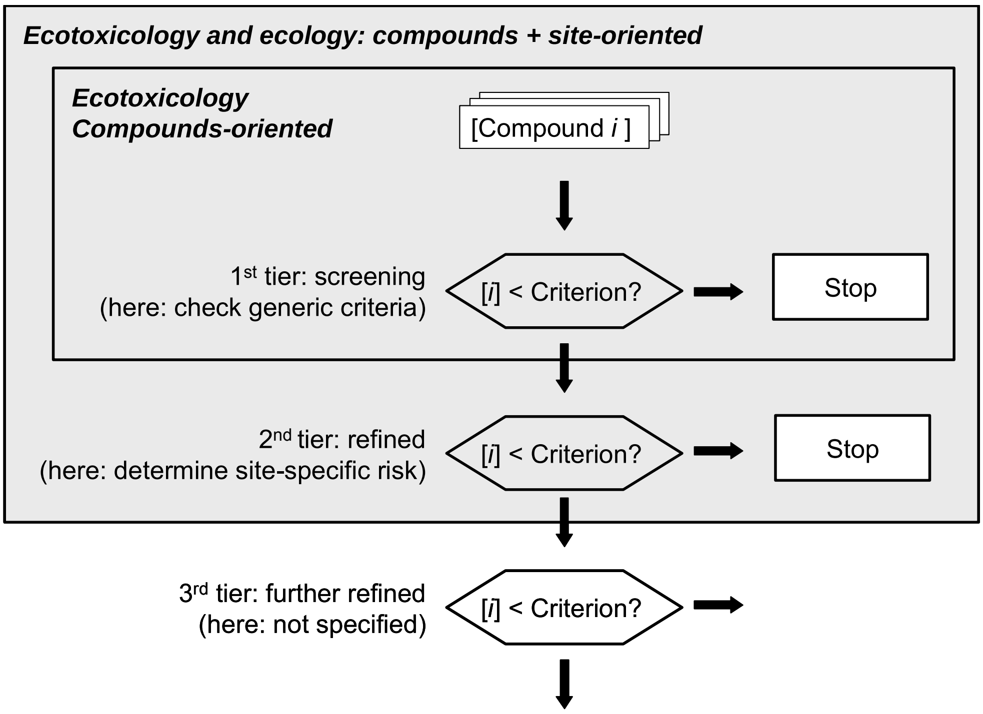

Figure 41. Operation of a tiered system in practice: flow diagram. When screening-level ecotoxicological assessments identify the option of unacceptable risks anywhere in the environment ("the generic ecosystem"), stepwise addition of data on the local mixture, the substrate characteristics and the receptor sensitivity, as well as ecological data, may show lower-then-expected local risks, and/or possibilities for reaching optimum ecological quality.

By using *the same risk assessment paradigm* for both chemical regulation in a narrow sense and for managing existing contaminations, it is possible to make a conceptual link between regulatory fields that have classically evolved separately. For example, by applying the same risk assessment paradigm, the compound-oriented approach in REACH can be brought in line with the dual approach in the Water Framework Directive, where the focus is on both chemical status and Good Ecological Status. This integration will be successful only when it is recognized that the problem definition of all regulations is different (e.g., preventive versus curative), that this translates into both similarities and dissimilarities of approaches followed in risk assessment, and that the dissimilarities should be logical and associated with the problem definitions. For example, using an SSD to derive a quality criterion follows the regulatory consensus approach of using NOECs to construct the SSD and to derive the HC5 as risk limit to define the regulatory criterion of MTR_{eco}, as originally described by Van Straalen and Denneman (1989). However, the SSD method, when applied in a retrospective assessment of seriously contaminated soil sites to rank them according to remediation urgency, could better use the SSD concept together with EC50s. This is related to the problem definition, namely to the fact that the study sites are highly contaminated. Using NOECs would result in chronic toxic pressure values that are expectedly varying between 50 and 100% (due to the Intervention Value being defined on the basis of a toxic pressure of 50%). The model would predict high toxic pressure values (>90%) for such mixtures (Figure 42). It is advisable to select a set of sites, ranging from clean to seriously contaminated, to derive guidelines for using the SSD model (und underlying data) in the derivation of risk management strategies.

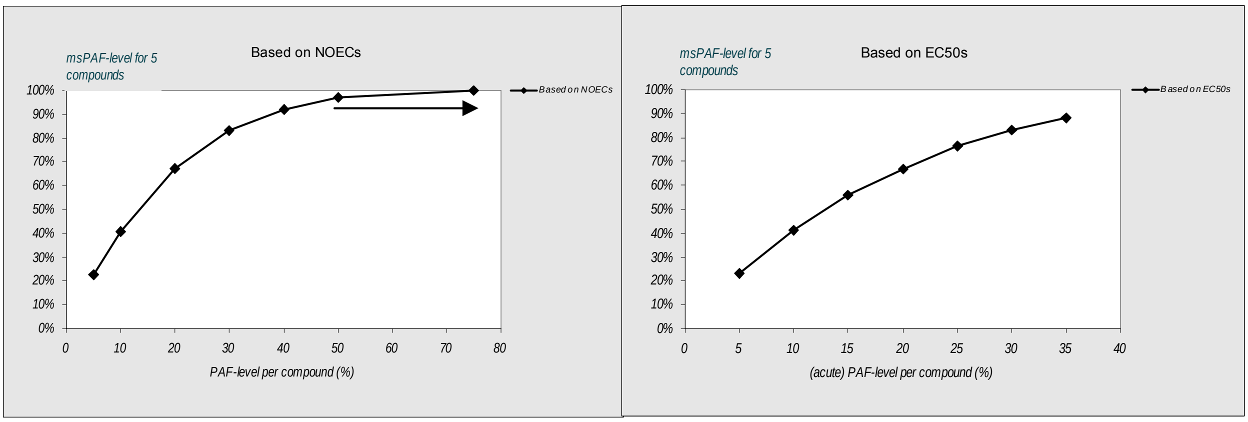

Figure 42. The dependence of the overall toxic pressure (msPAF) for an imaginary site, contaminated with 5 compounds at 7 exposure levels. (Left) Evaluation of the site, at 7 exposure levels whereby the chronic toxic pressure increases from 20% (5 compounds, with PAF per compound ranging from 5 – 75%. At a serious contamination site, the PAF per compound is by definition larger than 50%. Therefore, PAF-NOEC-assessment of such a site is not informative (arrow): exceedance of NOEC is predicted to occur for at least 97% of the species. A PAF-EC50-based assessment is still informative, even when there are 5 compounds with concentration acutely affecting 35% of the species each.

These reflections on the need for and implementation of second-tier assessment have resulted in the concept of a "toolbox for risk assessment". This concept will be discussed below in more detail. The aim of making a toolbox is to serve the various regulatory problem definitions with tailored approaches, founded on similar principles when possible, but diverging from them when needed.

The general issues for implementing the results of the SSEO programme in regulations (given many sites with existing diffuse pollution) are the following:

- there is a practical need for a tiered assessment approach that is founded on a uniform risk paradigm, to address site species variation in exposure and effects
- by developing and using such a tiering concept, there is latitude to establish linkages between regulatory approaches that up to now have evolved mostly independently, based on applying the same risk assessment paradigm, and
- this implies that a toolbox is needed to predict local impacts of existing site contamination, for the sake of improved management of diffusely contaminated sites (for which sanitation is not a realistic option).

The “what & how” of the toolbox is discussed in the next chapter.

31.7 Conclusions

The present attempt to summarize the scientific results of the SSEO programme suggested the following:

- the responses of biota in field conditions to diffuse contamination in the field are highly variable, ranging from little or no response at high exposure concentrations to large responses at low exposures
- the responses are determined by the combination of the compounds and their concentrations, the matrix, and the sensitivity of the exposed biota, evidently modulated by temporal and spatial factors, whereby:
    - the ability to prove exposure in field conditions is easier than proving effects.
    - the ability to find effects of diffuse pollution in the field, and to assign the effects to diffuse pollution, depends on the choice of measures of effect (sensitive, or insensitive), an appropriate analysis of other variables, appropriate observation designs and statistical study designs, and sufficient amounts of data
- failure to demonstrate responses of mixture exposure in field conditions cannot usually be interpreted as evidence for absence of responses, and

in a regulatory perspective:

- there is latitude to establish linkages between regulatory approaches that up till now have evolved mostly independently, possibly via adopting a tiered approach, and
- this implies that a toolbox is needed to predict local impacts of existing site contamination for the sake of improved management of diffusely contaminated sites.

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32 Validation of operational ecotoxicological models

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32.1 The toolbox concept and validation of toolbox models

Another aim of the present research programme was to determine the degree of validation of different models currently used for risk assessments in the regulatory context. Together with the integration efforts, this was a planned activity from the onset of the programme, expectedly resulting in creating the basis for a toolbox for risk assessment (NWO 1999; Posthuma et al. 2001). Model validation research should result in sets of clear model-use criteria, and thus also clear limitations on model use. As defined in Chapter 6, the validation studies were limited, and focused on confirmation, i.e. considering the similarity of model results and observations. During this process, the number of sites to challenge the prediction accuracy of the models was limited.

Given the tiered concept shown in Figure 40, it should be noted that a single model can have more than one confirmation status. When, for example, the SSD concept (in fact, the derived HC5) is compared to the NOEC of field ecosystems, it has often appeared that the HC5 criterion is sufficiently protective: the HC5 of a compound is often lower than the $NOEC_{Ecosystem}$; see Versteeg et al. (2006) for aquatic examples and Posthuma et al. (2002) for terrestrial examples. Some exceptions to this rule were shown in this report (Chapter 15), suggesting that the HC5 is *not* sufficiently protective in cases of acute exposure and multiple application of pesticides. Although some debate on the HC5 is thus possible, the concept is thus confirmed “for generic cases of chronic pollution” to be sufficiently protective. On the other hand, for the sake of ranking contaminated sites, affected species or the most toxic compounds within sites, the method also appears useful; for some purposes, it is enough that there is a general association between predicted toxic pressure and observed species loss, as observed in the eco-epidemiological analyses in Section 29.3.2. In the study, the SSD-based method shows that expected effects are larger at increasing concentrations. But at the same time, it shows that the SSD method is not sufficiently precise to predict exactly what will happen. The same result was found in a second study of the same kind (RIVM and UK Environment Agency, unpublished results). In other words, the concept of SSD can be considered (a) a confirmed model (based on various studies) for the derivation of quality criteria (except for some specific exceptions), (b) a confirmed model (based on few studies) for use as a ranking method, but (c) an imprecise model regarding the issue of exact prediction of kind and magnitude of consequences of diffuse exposure. In short, one model can have different confirmation statuses for different regulatory problems.

32.2 Models considered and generalized validation findings

Various models and approaches were studied in the validation case-study chapters of this report. The models pertained to

- exposure models, related to both the recognition of background concentrations and the dissolved concentrations available for uptake,
- an ecotoxicological-statistical approach, according to the SSD-concept,
- a population-dynamical approach,
- a food-web approach,

- a Case-Based Reasoning (CBR) approach, and
- an integrated ecological/ecotoxicological analysis of monitoring data for species and communities.

Each of the validation studies considered expert versions of the model under investigation, or expert use of those models. As a summary result, the validation studies did not reveal reasons why one of the studied models should *not* be adopted for inclusion in the toolbox for risk assessment. On the contrary, studied models are mostly part of officially adopted procedures, and therefore they should be part of the toolbox for that reason only. In this case, an official validation status has apparently been reached by the fact that they are used (and not discarded for reasons of apparently being useless in practice). Since they have reached this practical validity status, and are also ready for broader use, these models are the first candidate models to solve environmental risk management questions in higher tier assessments, and they may be adopted in the toolbox. The background information on the various models varies from scientific manuscripts that describe the model and its characteristics and use, to a complete package of user-friendly IT with supporting information (Posthuma et al., 2005).

Regarding the general results of the scientific validation studies, the following comments can be made. First, the set of validation studies is small. As stated earlier, the confirmation status of models increases with an increasing frequency of predictions that fit the observed field phenomena, and each of the models was only tested for one or a few situations. Second, there were few attempts to test various models on a single case. This relates in part to dissimilarities of datasets collected by different researchers (Chapter 30), in part to the fact that many studies were not designed to provide parameters for modelling, and to the fact that most studies were addressed with only one model rather than various models. Hence, a comparative test of the working hypothesis that “higher tier models more accurate predict field phenomena” could not yield strong insights on this matter (Figure 9). In one case, there was some tendency to support the conclusion that a higher-tier, complex mechanism-driven model performed better than other models. Given the context of a defined risk assessment problem, which can be either conservative or more precise, models of different validation status may be used. For example, the HC5 as derived from SSDs is, in general, sufficiently protective for ecosystem exposure to single compounds (it is apparently sufficiently conservative), and it grossly relates to biodiversity effects in the field (see Chapter 29), but it does not precisely define which species will suffer from which effects to which extent. It predicts *that* responses are likely, not *which* responses. Higher-tier models, like population models, do so (see Chapters 13, 14, 19 and 20). In these cases, the models worked as a magnifying glass on the field patterns observed in field assemblages of species, and only due to this, it was possible, for example, to separate the effects of flooding from the effects of diffuse pollution.

Looking at the compiled results of SSEO in another way (not according to “one hypothesized ideal model or approach”) shows another important feature of the SSEO programme. The programme has shown that a single problem (e.g., a site) can be analysed with various methods. These methods can serve as Line of Evidence (LoE) in a Weight of Evidence (WoE) approach. A WoE-approach uses the working hypothesis that all models and approaches are (despite conceptual complexity) still simplifications of true phenomena, and that this results in incomplete understanding and/or partly wrong assessment results. The WoE approach solves this problem by using various approaches in concert, i.e. various Lines of Evidence. In this sense, the SSEO programme results are like a formalized WoE protocol as is in use for the evaluation of existing cases of serious soil contamination: the triad approach (De Zwart et al. 1998; Jensen and Mesman 2006; Mesman et al. 2003). In this triad, various models and measurements are used in a tiered concept, to obtain insights into pollution effects, according to three Lines of Evidence: (1) the LoE-Chemistry (and msPAF), (2) the LoE-bioassays (looking at sentinel organisms exposed to field soil), and (3) LoE-ecological inventories (looking at local species assemblages and response endpoints).

It can be concluded that the use of the models has been helpful in unveiling the relationship between exposure of field biota to toxicants and the occurrence of effects. As also concluded by Pastorok et al. (2002) for an array of models, complete validation of models was beyond reach in 2002, and has still not been reached after the SSEO programme. However, steps have been made in the direction of understanding when to use, or not to use, the models (see below, for models separately). The use of a model should be considered only when the model type (output) fits the problem, and where the model precision (or its known bias to aspects such as over-prediction) fits the problem context.

32.3 Specific validation results and implications for the toolbox

32.3.1 Background concentrations and enrichment

The analyses of the exposure data have shown that models play a prominent role in the analysis of field effects. Exposure is, in a qualitative sense, one of the two major scientific analyses of the risk assessment paradigm (see Figure 8). In a quantitative sense, exposure assessment is absolutely not the least important of the two. Exposures differed greatly between sites, and these issues were in part uncovered and described by applying models.
- Model adoption: The model that is described in Chapter 7 distinguishes natural background concentrations from anthropogenic enrichment. The general approach of this model is founded in geochemical theories, in which field the approach is well established. When appropriate, the approach can be adopted to gain insight into the question whether a case of diffuse pollution is of natural or of man-made origin. This insight may be relevant for environmental management.
– Model adaptation. As yet, there is insufficient experience with the type of model applied in the context of an ecotoxicological problem definition. In cases where the model can be used, there is a need to adapt the model to the specific area and compounds under investigation.
- Abandoning the model. There is no conceptual or principal objective to generally discard the use of geochemical insights in the analysis of diffuse environmental pollution. However, it is common not to consider the distinction between backgrounds and enrichments in generic risk assessments, since in such cases the issue is not taken into account, or taken into account in a simplified way. For example, based on the concepts formulated for the so-called added-risk approach (Struijs et al. 1997), simplifying assumptions on background concentrations were made to derive, for example, criteria for metals (Crommentuijn et al. 2000).
- Model usage. Ongoing work in Dutch governmental research institutes focuses on the discrimination of natural backgrounds from anthropogenic enrichments. It is expected that this type of model will be used to further define the concept of “natural background concentrations”, as in the revision of the Dutch regulations on soil management and protection (VROM 2003; VROM 2006b).
- The model and the toolbox concept. When formal regulations require distinction of backgrounds and enrichments, there is no principal objection to adopting the model as part of a toolbox for risk assessment. Due to the fact that the model predictions relate to operationally extractable fractions, the toolbox could also adopt measurement techniques to measure enrichment rather than to deduce enrichment from various data sets.

32.3.2 Exposure modelling and physico-chemical availability

In addition to the motives given in the previous sub-section, this sub-section considers modification of exposure levels through modification of bioavailability. Exposures differed greatly between sites, and these issues were (in part) uncovered and described by applying models that consider bioavailability.
- Model adoption: The models that are described in Chapters 8, 9, 10, 11, and 12 all focus on describing the variability in uptake that depend on compound, matrix and/or species characteristics. Various models were improved on the basis of the data from the SSEO programme. In most cases, the models

or measurement approaches provided improved insights into the level of true exposure that is experienced in the field in diffusely contaminated systems. These insights are relevant for environmental management, especially when environmental management needs to make a priority selection for sites where remedial action is most needed. The priority of action, when related to expected impacts only, can be strongly modified by applying insights from exposure models. For example, Posthuma et al. (2006a) have used site-specific exposure models to correct for differences in local availability, to design a system to prioritize (within the large workload of sediments in ditches) which sediments can be spread on adjacent land with least expected impacts. Such a system was asked for by a specific regulatory workgroup (Kernteam Bagger & Bodem 2005), being confronted with an acute dilemma: contaminated sediment had to be removed for nautical reasons, but spreading the sediment on adjacent land in rural areas was suspect because of diffuse pollution.

- Model adaptation. There is a suite of exposure models, ranging from fully programmed and operational to described mechanistic conceptual models. Much work is going on in this area, ranging from research focusing only on the appearance forms of chemicals in the environment (abiotic) to approaches that also involve biota. Currently, for example, research efforts focus on the development and implementation of Biotic Ligand Models. Depending on the context of the assessment, risk assessors can select the most appropriate model. It is beyond the scope of this report to provide further guidance; specialized books on this issue (see for example Jager (2003) and references therein) are available.

- Abandoning the model. There is no conceptual or principal objective to generally discard the use of bioavailability insights into the analysis of diffuse environmental pollution. For generic risk assessments, however, correcting for availability differences between soils and sediments is often not implemented. This relates to the fact that availability can vary in time and space, which is considered to be problematic for general regulations (Wezenbeek et al. In prep.).

- Model usage. Bioavailability models are used frequently, but most often in the context of site-specific (higher-tier) risk assessments. Applying these models offers insights that are relevant for problem definitions with a prioritization aspect (see previous sub-section for further details).

- The model and the toolbox concept. For higher-tier assessments, all models for bioavailability correction could be part of the toolbox for risk assessment. For each of the models it should be considered which model is best suited conceptually to the problem definition of the risk assessment, and whether the use of that model is appropriate. The latter consideration should involve issues like limitations of use for each model, so that it is not used for cases for which it is not validated. Because availability can also be addressed through various measured extractable fractions, the toolbox could also adopt measurement techniques to measure available fractions, rather than to deduce availability from various data sets.

32.3.3 Exposure and food-chain modelling

The OMEGA45 model as applied in the present study is one of the few accumulation models with a longstanding history of development, calibration and validation. These are internationally accepted formulations that are fitted and tested to local conditions.

- Model adoption: For use in a protective context, validation studies show that the predictions of the OMEGA45 model are generally within the range of field measurements. However, deviations occur, and those have been the main focus of the present study.

- Model adaptation. The present study has demonstrated that bioaccumulation predictions can be improved substantially by using a more refined chemical model that accounts for bioavailability. Improvements were noted for several cases. Estimations of accumulation of polycyclic aromatic hydrocarbons in aquatic and terrestrial systems were improved substantially by taking strong sorption to black carbon into account. Likewise, accumulation of metals in terrestrial species was improved by using regressions that relate dissolved concentrations to total levels. In addition, accumulation factors of metals were shown to be concentration-dependent, causing order of magnitude errors in current risk assessment procedures that use fixed bioconcentration factors.

- Abandoning the model. The current studies have not revealed cases where there is a complete misfit between model predictions and data. However, modelling of essential metals such as copper and zinc needs specification of additional equations that account for active regulation. The work started in the present study will be continued in future projects.
- Model usage. For use in the context of deriving generic quality criteria, the use of the model is described in various publications. It is typically designed to be applied by specialists, answering questions put forward by the policy makers and local managers. Current dissemination efforts are directed towards implementation of individual equations or simplified versions of the model in environmental and water management.
- The model and the toolbox concept. OMEGA45 can be used within the domain as described in the separate publications. There are several ways to combine OMEGA45 with related models. Separate formulae may be used in other models, or OMEGA45 can be implemented in decision support systems. OMEGA45 itself combines exposure assessment with effect assessment at the level of populations and species. Moreover, equations for ecological and human exposure assessment are similar, allowing an integrated approach for different objectives in environmental management.

32.3.4 Species Sensitivity Distribution modelling

The analyses of semi-field effect data with the Species Sensitivity Distribution model suggests that SSDs can be used as a lower-tier model for both prospective (protective) and retrospective risk assessments of existing diffuse pollution.
- Model adoption: For use in a *protective* context, some validation studies show that the concept of HC5 does not always generate sufficiently protective criteria in specific cases (e.g., repeated spraying of pesticides causes acute effects at concentrations lower than HC5), although in the majority of validation studies known so far, HC5 offers sufficient protection at the community level of biological organization. When the model is used for quantification of local toxic pressure of single compounds or mixtures (prospectively or retrospectively), the model appears to predict *that* increasing effects are likely with increasing (ms)PAF, but not *what* exactly the response in a ecosystem will be. Figure 38 shows that an increasing value of predicted acute toxic pressure (msPAF) implies an increased loss of species, despite the huge variability caused by other stressors. The model can be adopted for both preventive regulation (for which it has already reached the status of "validation by use") and prioritization assessments. Examples of the latter are: prioritization of chemicals within mixtures (Harbers et al. 2006), prioritization in handling workloads of contaminated soils or sediments (Posthuma et al. 2006a), and prioritization of most likely affected species groups (as described in Posthuma et al. (2002a)).
- Model adaptation. The SSD model can be adapted, to make tailored variants for specific problems. For example, when additional information is available on specific Toxic Modes of Action of a chemical or a mixture, a refined assessment can be made to show which species are likely to be most affected by a given exposure. This follows from the effect of species selection on the shape and position of the "average" SSD (from all data) as compared to the shape and position of the various SSDs that can be derived when datasets are split according to Toxic Mode of Action (Jager et al. 2007; Posthuma et al. 2002a). Furthermore, the model can be used on the basis of various types of input. The classical use, for deriving quality criteria, is based on NOECs as input, and generates chronic toxic pressures as output. A chronic toxic pressure for 5% of the species has, in the past, been chosen as the Maximum Tolerable Risk level. Related to the problem of existing workloads of seriously contaminated soil and sediment, and based on available EC50 data, current work focuses on the derivation of standardized SSD-based methods to derive acute toxic pressure, based on SSD_{EC50}s. For practical use, there is a vast amount of data (>180.000 data entries) that can be used to derive SSDs, and in turn toxic pressures (Wintersen et al. 2004).
- Abandoning the model. The current studies have not revealed cases where there is a complete misfit between predicted and observed effects. The studies have, however, shown that the use of a simple SSD approach is not a panacea for all risk assessment problems. For various risk assessment problems it can be useful to obtain SSD-based insights, either as basis for decisions (both the quality criterion

based on HC5 as generic criterion for decisions as well as the (ms)PAF use to decide on further action), or as basis for targeted further investigations (for example, selection of the compounds contributing most to the toxic pressure in an area for uptake in a monitoring programme).

- Model usage. For the context of deriving generic quality criteria, the use of the model is described in various formal guidance documents. There are differences between various pieces of legislation (EU, national) in the exact implementation formats, including differences in the triggers or minimum requirements to use the model instead of fixed Safety Factors (for example, applied to the lowest NOEC). These partly historical, partly compound-group related differences could be factored out by making the guidance procedures fully data driven, rather than depending on fixed rules. An example of the latter type of rule is that at least *n* species from *m* taxonomic groups should be tested before an SSD can be used for derivation of quality standards. The availability of software, such as ETX (Van Vlaardingen et al. 2004) and OMEGA123 (Beek et al. 2002), and the functions programmed therein (like goodness of fit testing), would allow for more sophisticated usage rules. For example, instead of triggering on a minimum data input, a trigger for use can be a maximum width of the confidence interval of the SSD. This is suggested from a statistical perspective on the models' characteristics in relation to the problem definition of the assessment, because local risks are best managed when the *confidence interval* (not the amount of input data per se) is appropriate for the risk assessment problem. Although the interpretation of the model's results (msPAF values) has a *relative* rather than an absolute interpretation, this characteristic is not limiting the usefulness of msPAF as a surrogate parameter for a set of concentrations in monitoring programmes. Currently, various regulations prescribe that monitoring should take place. The study on the occurrence of fish species across Ohio rivers showed that the analysis of monitoring data can become possible when the potentially toxic agents are not separately analysed in the eco-epidemiological analyses (implying a considerable loss of statistical power with each compound added), but by the msPAF approach. This holds great promise for the implementation of the Water Framework Directive, for example, where monitoring programmes are currently being set up, and where spatially explicit data analyses (and identification of deviation from Good Ecological Status and causation) will be needed in the near future.

- The model and the toolbox concept. When SSDs are considered as models to be adopted in the toolbox for risk assessment, it can be stated that the model has been scientifically scrutinized (Posthuma et al. 2002b), that an array of validation studies have been made (broader than the ones shown here) and that the model is available for broader use, supported by both the model functions in ETX (Van Vlaardingen et al. 2004) and by easy data access through the e-toxBase (Wintersen et al. 2004). In fact, the calculation of toxic pressure has recently been adopted in the toolbox for site-specific risk assessment according to the new Dutch soil policies (Posthuma and Wintersen 2007; RIVM et al. 2007), in which it is used for both (a) improving insights in the level local ecological stress (in addition to classical risk indices) and (b) in the criterion with which sanitation urgency is determined.

32.3.5 Population dynamical modelling

The population dynamical modelling (e.g., PODYRAS) that was used in various chapters of this report was very helpful to disentangle the effects of toxic stress from other environmental stress. The model made it possible to study in detail the effects of flooding on population viability and indicated that earthworms in frequently inundated sites adapt to the local inundation regime by maturing at an early age. This biological adaptation, in combination the direct effect of flooding (following a flood the number of earthworms is greatly reduced), has a large influence on the total abundance and density of earthworms. The last two are in general the parameters which are chosen as measures of effect to assess the possible influences of toxicants in the field. If adaptation indeed is a general phenomenon, we may expect that abundance and density primarily reflect the influence of flooding, and that effects of toxicants are not easy to visualize. Adaptation to flooding proved to be a general phenomenon, as shown in Chapter 19, by analysing a large data base on earthworm data in not-polluted floodplains. We therefore conclude that the fact that toxicants did not prove to have an effect on abundance and density in published studies was a false negative that must have resulted from the fact that flooding induces

statistical noise in the abundance and biomass data. Without the possibility to apply a mechanistic model, and the idea that adaptation is a general phenomenon, we could not have combined various relevant data sets from the SSEO programme with studies from other origins to increase the sample size of the study. This approach has proven useful to clarify the effects of toxicants in floodplains on earthworm populations.
- Model adoption. The model was adopted to assess the simultaneous effect of flooding stress and toxic stress, and to disentangle the effects of both stressors. Given the association between the occurrence of earthworm populations (those that survive both flooding and toxicant stress and those that apparently did not), the modelling seemingly operates as “magnifying glass for toxic effects” in the case of multiple stress on populations.
- Model adaptation. The model was adapted to assess the effect of flooding and to include space explicitly.
- Abandoning the model. For all studies reported in this report, the model behaved well; it gave insight into ecological phenomena and did a good job of predicting the effects of toxicants, which was validated with field data.
- Model usage. The model is very useful to assess the site-specific impact of toxicants on earthworm populations (or populations of species in general, provided that biological base data of the species are known), especially since it can also address the simultaneous effects of other stress factors.
- The model and the toolbox concept. PODYRAS has been scientifically scrutinized by Klok and De Roos (1996). It can be adopted as part of the risk assessment toolbox, and it can be applied when appropriate.

32.3.6 Food web modelling

The use of food web models in determining the effect of field-relevant toxicants has mainly focused on bioaccumulation in the current research. An additional use of food web models is the prediction of the impact of toxicants on the interactions between species and functioning of ecosystems. Although sophisticated effect models are available for predicting the dynamics of pesticide pollution events (e.g., CATS models, Traas et al. (2003), this class of models is time consuming to build, calibrate and verify. These models can be used for specific case studies, but generic effect predictions for terrestrial food web are preferably made using a generic food web model (see Chapter 14).
- Model adoption, model adaptation and abandoning the model. Generic food web models such as SimpleChain can be verified by comparing model results to semi-field experiments. The limited experience with this class of models indicates that the model correctly predicts that sensitive species disappear under chronic toxic stress, in a realistic range of concentrations. Similar observations or species-dominance changes, without the use of the model, were found in the Ohio eco-epidemiological study (Chapter 29). More extensive validation is needed to prove more general applicability. The merit of the model is in explaining why a slight loss of biodiversity often goes unnoticed; the ecosystem function is usually affected at higher toxicant concentrations, which is a highly non-linear process as compared to the loss of individual species.
- Model usage. The SimpleChain model was applied with the following principles:

1. The ecosystem is described by a number of parallel food chains, which together form the entire ecosystem. The number of chains is variable and can be set by the user. This determines the number of species within each functional group.
2. Each food chain is composed of a microbial assemblage, a detritivore and a carnivore.
3. The species sensitivity of each functional group is described by an SSD, based on literature data for a specific chemical.

The model predicts the response of the parallel food chains to the toxic stress, while still calculating which species in the parallel food chain survive in the struggle for resources. Species that are not efficient or weakened by the toxic stress are out-competed by stronger species. The model easily shows that toxic stress can be masked by ecosystem redundancy, where less sensitive species take over from sensitive species and keep the ecosystem going.

- The model and the toolbox concept. The SimpleChain model could be considered for adoption in the toolbox for risk assessment. The model can predict field concentrations corresponding to specific mortality probabilities, or estimate the probability of biodiversity loss based on measured field concentrations. The model is freely available but needs an easier interface.

32.3.7 Case-Based Reasoning (PERPEST)

The current papers and studies performed with PERPEST indicate that the approach can be used to translate measured and modelled concentrations of pesticides into ecological risks. The main advantage of using the model over laboratory derived data is that it decreases the uncertainty of extrapolating to the ecosystem level. Because the effects data are based on mesocosm studies, there is still the uncertainty of extrapolating the mesocosm data to the field. The benefit of the model is not only that it removes some uncertainty by including experience from other pesticides, but also that it quantifies the uncertainty by taking a probabilistic approach and providing uncertainty limits to predicted values.
- Model adoption, model adaptation and abandoning the model: Since the model is based on the highest-tier data available in the risk assessment of pesticides, it is difficult to validate or reject the outcomes of PERPEST. It is, however, of great importance that the results of the model are interpreted in the light of their validity (see below, model usage).
- Model usage. When the model is applied to a particular case, some assumptions are made:

1. The cases upon which the prediction are made are representative for the question case with respect to ecosystem structure and functioning and exposure regime;
2. The calculations of dissimilarity and transformation, standardization and weighing of variables used in the model are adequate for making predictions;
3. The number of cases present in the model is sufficient for making a prediction.

The major sources of variation left are the variability of responses between different ecosystem types and the question of how representative the structure and functioning of the model ecosystems and exposure regime used in the experiments incorporated in the model are for the question case. It has been argued by some authors that threshold values and direct effects observed for the same compound were very similar in different aquatic ecosystems. This variability in response is, together with the variability in exposure regimes, incorporated into the confidence limits of the predicted probability, and is thus explicitly expressed in the output of the model.
- The model and the toolbox concept. When the PERPEST model is considered as a model to be adopted in the toolbox for risk assessment, it can be stated that the model has been scientifically scrutinized (Van den Brink et al. 2006b) and that the model is freely available for broader use via www.perpest.wur.nl.

32.3.8 Eco-epidemiological analyses

All SSEO-field effect studies imply that the “signal” of the ecotoxicity of mixtures needs be unravelled from the “noise” attributable to the common ecological variation. The basic “model” to follow here involves variants of the postulates of Koch, which were designed to “prove” that disease was caused by a certain microbial agent. In the present case, these postulates were worked out by using an ecotoxicity model (the SSD, see above) in conjunction with ecological modelling and statistical analyses. Note that eco-epidemiological analyses will – in type of results – never provide “proof of causation”; they will conceptually only provide plausible association (by statistical associations).
- Model adoption. There is a growing regulatory need for meaningful analysis of monitoring data sets. Related to various regulations (e.g., the EU Water Framework Directive and EU-REACH), there is a specific need to consider both the exceedance of generic quality criteria for a set of compounds, as well as the need to address whether an environmental compartment fits the criterion of a Good Ecological Status. Linkage between ecotoxicity analyses and ecological characteristics of realistic environmental compartments is needed. As an example, the EPC model provides one of the foundations for further addressing these needs. However, since eco-epidemiological analyses *always* yield statistical outcomes rather than proof of cause-effect relationships, critical appraisal of approaches is essential. Currently,

the results of the EPC approach are therefore the subject of a comparative study in which another eco-epidemiological model is used to analyse the same data set (Posthuma et al. 2006c).
- Model adaptation. Eco-epidemiological modelling is still in its infancy regarding the analysis of low-level mixture exposures as part of a complex set of stressors and field phenomena. It is likely that the available models will be adapted further, as experience grows.
- Abandoning the model. The current analyses have not provided evidence that the approaches followed so far should be abandoned.
- Model usage. It is to be expected that regulatory agencies will, in the near future, require the use of eco-epidemiological models in which the role of toxicant mixtures is explicitly addressed, in order to analyse the data generated in large-scale monitoring programmes. The EPC-approach has some highly desirable characteristics. For example, it considers that the ecological condition of each site in reference conditions can differ from other sites, it considers the mixtures by using a single aggregate parameter (msPAF), which greatly improves the statistical power of the analysis, and it present results in a format that is easy to understand (risk communication). Especially in the context of the Water Framework Directive, the focus is on reaching a Good Ecological Status, which now requires planning monitoring schemes and collecting data, but over the longer term, methods (such as the EPC method) to analyse those data will be required to obtain information on the ecological status.
- The model and the toolbox concept. Due to the specific nature of monitoring data sets, approaches like the EPC approach will not easily be adopted as modelling tool in a toolbox. However, the stepwise analysis that is followed could become a standard approach, and thus a sort of protocol. It is recommended to specifically develop eco-epidemiological techniques, because (for example) the Water Framework Directive specifically focuses on reaching Good Ecological Status, whereby deviation from this status needs be investigated to define appropriate Programmes of Measures. Within a protocol for eco-epidemiological analyses, the use of some toolbox modules, such as SSDs, is crucial.

32.4 Towards a toolbox for site-specific risk assessment

32.4.1 Increasing variability in risk assessment questions

In the project proposal for the model confirmation and SSEO integration project (Posthuma et al. 2001), the word "toolbox for risk assessment" was introduced. From the Abstract: "*A decision-support toolbox for environmental management purposes is assembled from existing ecotoxicological effect models. Using the information and insights generated in [other] SSEO projects, the available models are tested, calibrated, validated and adapted where necessary*". The introduction of this term is logical due to the current possibilities offered by information technology and from the increasingly complex questions posed by regulators. The substance-oriented preventive risk assessment issue, which requires the derivation of a "safe concentration level, protecting a generic ecosystem against a maximum-allowable impact per compound" is extended by questions posed in the context of compartment-oriented policies (water, sediment, soil). A site-specific problem definition is "does the local mixture of toxic compounds influence a local ecosystem, and if so, what should I do as an appropriate management measure (clean-up, other risk management)?". Or, in the case of a large workload of slightly to highly contaminated sediments that must be handled: "how should I prioritize between sites for sediment management, given the differences between sites regarding toxicity, ecosystem type and necessity related to nautical reasons". In other words, more diverse, and sometimes repetitive, answers are needed to realistic risk management questions that are outside the original scope of the criteria-setting risk assessment. Especially, the Dutch government asks for not only "environmental effects assessments" when new policies are being developed, but also for "cost-effects of proposed regulations" (EZ/VROM/Justitie 2003).

32.4.2 Motives for the implementation of a toolbox for risk assessment

The motives for developing a toolbox for risk assessment are, in summary, that:
Regarding the problem definition of realistic environmental problems

1. diversification of problem definitions: risks need be determined not only per compound for the derivation of quality criteria in worst-case conditions, but also for an array of possible problem definitions;
2. more specific problem definitions: risks need not be determined for a generic ecosystem, but for a known ecosystem, for which both the local mixture, the local substrate, and the locally exposed biota are known;

Regarding quality aspects of addressing the problem

3. improved versatility: where the substance-oriented risk assessment was (in principal) done once and by experts, and resulted in a single value (the quality criterion), the new types of question ask for the possibility to run risk assessments tailored to the risk management problem definition
4. improved efficiency: whereas the substance-oriented risk assessment was (in principal) done once and by experts, and resulted in a single value (the quality criterion), the new types of question ask for an efficient risk assessment process, which can be run repetitively, when needed, by third parties;
5. reproducibility: each tailor-made risk assessment, when used in a regulatory context, should be reproducible, i.e. "captured in software", which in turn implies the use of at least three IT modules, one for exposure assessment, one for sensitivity assessment and one (combining both) for risk characterization (see Figure 8)

Regarding technical aspects

6. information technology makes toolbox composition, use and version management feasible, especially through web-based approaches.

These motives are also encountered internationally. Over the last few years, the concept of a "toolbox for risk assessment" has thus been mentioned in various contexts, for example.:

- Solomon et al. (In press); this author organized an international workshop on current methods for the effect characterisation of chemicals, and noted that for each problem, multiple solutions (models and/or measurements) exist, ranging from simple and conservative to more complex and realistic, and that these methods can be organized according to a tiered approach, in the format of a toolbox for the characterization of environmental effects of chemicals
- Carlon (2005); a European project called HERACLES is currently working on international compilation of approaches in risk assessment in the European Union;
- VROM (2006b) and Posthuma and Wintersen (2007); currently, the Dutch soil policies are being renewed, which involves the production of a Toolbox for soil quality assessment. This toolbox is currently ready for publication and use via the web (URL: www.risicotoolboxbodem.nl). It is meant, in its present form, to support the derivation of so-called Local Maximum Values (criteria for judging soil based on site-specific policy developments) and to increase insights in the meaning of local diffuse soil pollution. Based on the web-portal concept, it also serves as a link to other tools, that will be added soon. All web tools are meant to be based on repeated application of risk assessment tools (like an exposure assessment model) whenever possible. In this way, knowledge is efficiently applied, and results are reproducible and internally consistent and comparable.

32.4.3 Expectations about and criteria for toolbox design

Based on the trends that were observed nationally and internationally, it is to be expected that the toolbox concept will be evolving further in the near future, both nationally and internationally. For this reason, it is worthwhile to elaborate on some design criteria. Both scientific and practical issues can be mentioned.

An important issue is versatility. The toolbox should be composed so that the increasing variety of assessment problems can be addressed with appropriate approaches. Such approaches can consist of both modelling and measurement, or a combination thereof, to address both exposure and effects assessment aspects of risk assessment (see Figure 8). A second issue is tiering. Since there are various models and approaches for a single problem (differing in complexity, precision and data need), the available approaches should be used in a tiered system. Posthuma et al. (In Press) have suggested a generalized tiering system based on an overview of current extrapolation models in ecotoxicology. Another issue is the standardization approach to repetitive problems. In the same way that the derivation of generic quality criteria is defined through standardized guidance documents, other repetitive tasks should be addressed by standardized approaches. In the case of SSDs, this is possible by adopting, for example, standard sets of ecotoxicity input data for each compound at the EC50-level, whereby sets are revised in regular intervals. From a practical point of view, it is to be expected that additional assessment modules will be added, tailored to novel problem definitions or situations, and that this implies that version control is needed. In some cases, the results of different toolbox modules may be combined, according to the Weight of Evidence principles described earlier. Furthermore, it suggests that different, evolving versions need be offered to the users. This means that a web-based management approach is needed for the toolbox. The models and approaches in the toolbox should have technical and scientific support, the latter in the format of scientific publications describing the merits of the techniques, and this information should encompass issues like limitations on the use of models and approaches. Finally, it is important that the toolbox output provides a comprehensive reporting of risk assessment results. This was stressed in a recent project, and specific recommendations are given (see www.eufram.com).

32.5 Conclusions

The analyses on validation of models have yielded the following conclusions:

- Models are helpful in disentangling the field responses of biota to exposures to mixtures of contaminants
- Next to models, measurements may also be applied, or models and measurements may be applied together in a Weight of Evidence approach
- Models pertain to exposure models, especially regarding the recognition of natural background concentrations and regarding the availability of compound fractions for uptake by biota
- Models are, by their nature, a simplification of reality; models therefore need not be necessarily good and true representatives of sets of phenomena that occur in the field; as a sketch, a good model “should cover 80% of the field phenomena based on 20% of aspects of truth covered in a simplified way”
- For most of the scientific phenomena, there is more than one model available; depending on the goal of the assessment, the most appropriate model may be chosen, i.e. for the protective context (setting generic quality standards) simple models are needed (for many compounds, and based on little information), and these models may bias towards conservatism; for site-specific assessments, but based on the same risk paradigm, more complex models can be needed, which are increasingly focusing on local site and biota characteristics, and which are less conservative (and more realistic).
- Most existing models have been developed by individual scientists for individual problems; arguments on efficiency and reproducibility suggest the development of a toolbox for risk assessment

- The toolbox for risk assessment should be as versatile as the questions posed to regulatory ecotoxicology. A toolbox should contain approaches varying from lower-tiered simple and conservative models (that work for the multitude of compounds), models that allow for site-specific assessments, and models like Weight-of-Evidence methods (that apply local measurements and bioassays).
- Regarding its contents, the scientific toolbox should be designed according to two major principles: (a) it should allow for incorporation of a versatile set of tools that can be ordered according to (b) a tiered approach. Both are relevant in view of the diversification of questions on the one hand and internal consistency of a tiered system on the other.
- When used in regulations, guidance should be provided on standardized ways to use the toolbox for similar sets of problems, for example determining the handling of contaminated sediment or determining clean-up needs and urgency.

33 Dissemination and regulatory issues

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33.1 Overview

The major aim of the research for this report was the dissemination of SSEO results to the regulatory context. As stated in the general introduction (Chapter 1), diffuse pollution is addressed not only in regulations focusing on chemicals, but also in other ones pertaining to specific compartments (water, sediment, soil) or specific protection of biota (species or communities, including their habitats). Thus, a complex regulatory situation exists for diffuse pollution: (1) there are vast areas, or soil and sediment volumes, with diffuse pollution; (2) there is an array of relevant regulations; and (3) the research demonstrated a variety of responses that are not predictable from exceedance of quality criteria.

In this complex situation it is a major task to derive “ways forward”, for all regulations – separately and together – as well as for the authorities responsible for the management of diffusely contaminated sites. Specifically, the latter are confronted with public concerns triggered by cases of criterion exceedance, and they especially require approaches and tools to solve practical problems. Such issues become urgent for them primarily in cases where changes in site use are planned. Such changes may imply changes in the availability of toxic compounds, whereby it is often expected that changes in land use imply increased physico-chemical availability (and probably effects). National and international authorities are, however, also confronted more intensively with diffuse pollution. For example, the EU Water Framework Directive emphasized a dual approach. First, according to the classical compound-oriented approach, water bodies must have good chemical status (no exceedances of quality criteria), which involves looking at various chemicals and point emissions. Second, and additionally, water bodies must have or reach Good Ecological Status, which requires an added focus on, among other things, diffuse pollution.

From the local to the national level, authorities are thus confronted with a tension between the use and interpretation of generic quality criteria; they are also confronted with the site-specificity of impacts, which were highlighted in the overall results of this programme. This chapter describes options for addressing the problem of diffuse contamination, both from the general perspectives for improved national and international regulations and from the practical point of view of responsible authorities confronted with diffuse contamination at a site or a number of sites.

33.2 Possible implications for regulations: chemical-orientation

33.2.1 Prevention and EU-REACH

The chemicals policy in Europe, including the Netherlands, is currently specifically based on the recently adopted REACH regulation (see below for acronym definition). Besides to this regulation, specific legislation is in place for pesticides, as well as for specific compound groups other than pesticides, such as pharmaceuticals and cosmetics. Furthermore, additional legislation applies to risk management of slightly contaminated ecosystems and remediation of seriously contaminated ecosystems. In this sub-section, the focus is on the preventive, risk management, and curative approaches, in that order, and on the relevance of the findings of the SSEO programme for those approaches.

The new EU regulatory framework for the Registration, Evaluation, restriction and Authorisation of Chemicals (REACH) entered into force on June 1, 2007. Important aims of this regulation are to improve the protection of human health and the environment through a simpler, more efficient regulation and to generate more information on the properties of chemical substances. The regulation is founded on the principle that producers of chemicals are responsible for the required assessment of data, data evaluation, risk assessment and risk management. The REACH process was started because previous procedures had resulted in too few risk assessments (for only less than 200 compounds), while more than 100,000 compounds are known (Commission of the European Communities 1990). Under the REACH regulation, industry has to register about 30.000 substances in a period of 11 years after entry into force. The registration process is applied in a stepwise fashion, depending on the production volume in different tonnage ranges, and on the hazardous properties of the substance. High production volume chemicals (> 1000 tonnes per annum) will need to be registered early, as will substances of high concern: carcinogenic, mutagenic or reprotoxic substances (CMR substances) or potentially persistent, bioaccumulative and toxic chemicals (PBT substances). The standard information requirements on hazardous properties also depend on production volume, where more requirements need to be met with increasing tonnage.

The assessment of toxicity of chemicals (hazard assessment) is the most relevant aspect with respect to the focus of the SSEO programme. It involves, for example, the determination of a PNEC (Predicted No Effect Concentration) for chronic exposure. The integration of the SSEO study data suggests that the current approaches to derive PNECs (as protective limits) appropriately serve their regulatory goals in the majority of cases (known exceptions are cases of repeated acute exposure, which as common for pesticides, see Chapter 15). This means that the PNEC that is derived in the hazard assessment is often sufficiently protective for maintaining structural and functional integrity of natural communities. This has been observed both outside and within the SSEO programme.

Regarding the single compound point of view, the results of SSEO studies do not suggest that major changes are needed in the methods of derivation of PNECs that serve as operational protection criterion. A major directional change, for example a general increase of PNECs due to the use of lower safety factors in their derivation, is not warranted by the SSEO results, because of the possibility that sensitive ecological cascades induce effects on target species (see Chapter 28). Apart from this field evidence, model results also suggest that sensitive cascades may exist (see Chapter 14). Toxicant effects may be masked, for example by flooding (Chapter 20), but ecological interactions like predation and competition may result in a quicker decline of some groups than expected from NOEC exceedance alone (Chapter 14).

The REACH approach does not look at field effects of ambient mixtures that occur as a consequence of compound manufacturing and use. REACH takes primarily a compound-by-compound and producer-by-producer approach. In a risk assessment process called substance evaluation, governmental authorities have to assume the responsibility to assess the cumulative effects of chemicals that could cause risks on an EU-wide scale. The SSEO studies and some extended studies (such as the butterfly study and the eco-epidemiological analyses in Chapters 22, 28 and 29) have shown, however, that in some cases mixture effects can be discerned from natural background variability, even at moderate to low field exposure concentrations – provided that the observation design is appropriate and of sufficient magnitude. The presented evidence for mixture effects suggests that the overall target of REACH (protecting humans and the environment from adverse effects) may not be fully covered by a substance-by-substance approach. Major scientific variables that influence toxicity have been studied in SSEO and elsewhere. Issues were identified regarding aspects of, for example, bioavailability, mixture toxicity and sensitivity differences between species or between processes.

In evaluating the effectiveness of REACH, scientific methods could be used to address the issues identified in SSEO. The specific emphasis should then be on the following:

a) Possible effects of mixtures of chemicals. Within REACH, authorities can suggest restricting the use of certain chemicals. If a group of chemicals with similar toxicological properties has a cumulative toxic effect unnoticed in the registrations for each substance, restricting the use of some substances within that chemical group could be an option if risks need to be controlled at a community wide level.
b) Monitoring the overall toxicity due to remaining emissions that result from the scope of the "per chemical" approach. This could be an addition to methods to evaluate the effectiveness of REACH in controlling or reducing emissions of harmful substances.

Some of the models studied in the SSEO programme can serve REACH-related assessments. In a recent study, a successful attempt was made to quantify the local toxic pressure of mixtures of high production volume chemicals at prolonged emissions at current rates, in order to identify (a) whether a real problem could be found that corresponded to the modelling results, and (b) which compounds could be assigned a major role in causing probable impacts. A similar study was made on pesticides. The studies were executed and described by Harbers et al.(2006) and Van Zelm et al. (Subm.). Based on production volumes, emission rates and fate assessments (the exposure analysis-side of a risk assessment), and based on hazard data (in this case: SSDs for each compound), these authors calculated the probability and magnitude of ecological effects in aquatic ecosystems that would result from the cumulated emissions of each substance. In these studies, all variables of the risk assessment were taken explicitly into account to represent the level of confidence that we have in "knowing the relevant variables".

This type of approach can be used by regional governments or water authorities (see the sub-section on the EU Water Framework Directive below), to address the problem of cumulative toxic stress from multiple compounds from multiple sources (e.g., either distinct production facilities or widely dispersed diffuse emissions). GIS presentation of net toxic pressures expected for river catchments, for example, are possible, satisfying the needs formulated by Bradbury et al. (2004). The same types of methods might be used in case of local concerns with specific compounds, for example, with compounds having a production volume that is too low to be addressed by REACH. For these compounds, assessments triggered by specific local concern might identify the true cases of concern.

The SSEO studies focusing on field effects are relatively "distant" from chemical regulations such as REACH. However, REACH is addressing only to a limited extent the cumulative effects of chemical releases that lead to diffuse contamination. For that aspect, it is advisable to:

- further develop approaches to enable recognition of problems of cumulated emissions by regulatory authorities
- consider linkage across regulations, specifically between REACH and the Water Framework Directive and the Soil Strategy, so as to avoid conflicting rules and approaches.

33.2.2 Pesticide policies

The work presented by various authors (Maltby et al. 2005; Roessink et al. 2006a; Van den Brink et al. 2006a; Van den Brink et al. 2006b) validates the use of the SSD concept in the risk assessment of pesticides by comparing its output with effects observed in semi-field experiments. These results indicate that if the exposure regimes match, sensitivity of sensitive species as estimated in the laboratory and described by SSD can be used for predicting direct effects in the field. Concerning the use of SSD, the Technical Guidance Document on Risk Assessment (Commission 2003) specifies that SSDs (when used in the protective context) should only be constructed from no observed effect concentrations (NOECs) resulting from long-term/chronic studies, by using the most sensitive endpoint for each species or the geometric mean of multiple endpoints. Our findings illustrate that SSDs generated using acute EC50s can also be used in case of a short-term exposure regime.

The SSEO findings indicate that more work should be done to evaluate the effects of chronic exposure of pesticides on aquatic communities. Especially because of the integration of this risk assessment framework with the Water Framework Directive, it can be expected that more emphasis will be put on low and chronic exposure. While several studies have been performed on this in Wageningen (Hartgers et al. 1998; Kersting and Van den Brink 1997; Van den Brink et al. 1997; Van den Brink et al. 2002b; Van den Brink et al. 2000; Van den Brink et al. 1995), the effects of these concentration profiles at the community and ecosystem level has received little attention outside of this research group. The SSD concept can only be used when recovery is not included in the risk assessment; this is because recovery is not taken into account by SSD. Within the risk assessment of pesticides, small effects are, however, considered acceptable if recovery takes place within an acceptable time window. If recovery is of concern, semi-field experiments and/or meta-population models are recommended (e.g. Klok et al. (2006a) and Van den Brink et al. (2006a). The use of metapopulation models is an interesting option to extrapolate results from laboratory and semi-field experiments, in both time and space, to the landscape level. The main advantage of these types of models is that not only that the toxicity of the pesticide is taken into account, but also the life-cycle characteristics of the species and the ecological infrastructure of the landscape. This provides realistic recovery times following perturbation, something that is not possible using currently available experimental and modelling tools. Furthermore, eco-epidemiological analyses are possible, provided that pesticide exposure data are available on a landscape scale. Some experience on this aspect has been acquired by applying the analysis technique to data from England and Wales (De Zwart et al., in prep.).

33.3 Possible implications for national regulations: compartment-oriented

33.3.1 Possible implications of SSEO findings for water policies

National and international directives for water management generally aim to improve actual risk assessment by using up-to-date toolboxes. The EU Water Framework Directive for instance, requires investigative monitoring if ecological objectives are not met. This has resulted in the development of instruments that link physical-chemical stressors to biological responses, for instances in the EU project REBECCA. The OMEGA123 and SSD models, for example, have been selected as tools for determining the impact of toxic stress on lower species groups. Although top predators are not yet part of the monitoring obligations for water systems, accumulation has been identified as a missing factor to be addressed by OMEGA45 type of equations.

Similar developments apply to local risk assessment, often carried out in the framework of Dutch policies such as “Room for the River” and “Water Management in the 21ste century”. Sites identified as polluted by simple quality standards and standard soil or sediment correction factors are often studied in detail, taking into account chemical and biological variation in bioavailability. Here too, OMEGA45-type equations and transfer functions are being used for more refined assessment (Hendriks et al. 2001; Vijver 2004).

33.3.2 Possible implications of SSEO findings for soil policies

33.3.2.1 Risk management of diffuse pollution

The findings of the SSEO programme have important implications for a relatively novel approach: risk management of diffusely contaminated soils and sediments. Previous regulations mainly discriminated between clean soils and sediments (below the Target Value) and cases of serious contamination (“*ernstig geval van bodemverontreiniging*”), leaving the diffuse contamination cases rather undefined. The ongoing soil policies modernization in the Netherlands (*Besluit Bodemkwaliteit* (VROM 2006b)) explicitly recognizes the existence of vast areas and volumes of soils that are neither clean nor “seriously contaminated”, for which remediation is neither required nor urgent. For these soils, a system of soil classification has been designed for national use based on generic risk considerations including different types of soil use, in addition to a system for local soil policies. In the latter system, local issues – like an increased local background concentration that is not risky for present or planned soil use – can be incorporated into the local soil policy plan. In the latter case, it can for example be acknowledged that – given the high sensitivity of sheep to copper exposure – a copper-contaminated soil can be used for many purposes, except rearing sheep. In other words, site-specific risk assessment is a novel part of both the national regulation and the possibility to make a local soil management plan. To allow local soil managers to address risks in a site-specific way, the national government has recently provided a toolbox for site risk assessment and soil-use-specific risk assessment; URL www.risicotoolboxbodem.nl (Posthuma and Wintersen 2007; RIVM et al. 2007). The main web tool is the module “determination consequences Local Maximum Values”, in which:

- “Local Maximum Values” are those compound concentrations that will be established as local concentration criteria for toxic compounds, and that serve as criteria for soil and sediment management; to acknowledge specific local conditions, these values can be higher or lower than the generic class values.
- “determination of consequences” is the result of site-specific risk modelling, in which proposed levels for the Local Maximum Values are considered, that is: tested whether they would cause unacceptable risk for current or planned local soil use.

The results of SSEO – and the recognition that site-specific risks can differ greatly from generic potential risks – were obtained concurrent with the definition of the major outlines of the new soil policies.

In view of the vast areas and volumes with diffuse pollution, it is advisable to develop a toolbox for risk assessment, not only for soil (as recently done), but also for water and sediment problems. This toolbox should be useful for site managers, to help formulate risk management plans that are both environmentally appropriate (offering sufficient protection given e.g. local soil use) and cost effective. The toolbox should not only focus on risks, but also on the behaviour of compounds in the environment. For example, diffuse contamination of topsoil might lead to vertical transport of contaminants and violation of groundwater protection targets. The toolbox should thus be formatted with a systems approach rather than a compound-oriented approach.

33.3.2.2 Curative policies

The increased emphasis on site-specific risk management (previous sub-section) evidently also applies to curative policies, i.e. those regulations that are in place for the highest-risk sites with high contamination levels. However, contaminant concentrations at those sites are usually not called diffuse contamination, but more formally “a serious case of soil pollution”. Site-specific assessment methods have been in place for a long time (e.g., for contaminated soils (VROM and Van Hall Instituut 2000) and for contaminated sediments (VROM 1997)), and are currently being reviewed and replaced, in part based on the new insights into modelling and site-specific risk assessment (e.g., (VROM 2006b)). It is advisable to develop the toolbox for site-specific risk assessment in such a way that all cases of contamination (being diffuse or serious) can be handled efficiently; whenever appropriate, similar approaches should be used for exposure and effect assessment.

33.3.2.3 EU-soil Strategy

In response to concerns about degradation of soils in the European Union, the European Commission published a Communication “Towards a Thematic Strategy for Soil Protection” in April 2002. Soil contamination was one of the themes that was considered by a technical working group. The Thematic Strategy for Soil Protection was adopted in September 2006. The Strategy contains a Communication (COM(2006) 231), proposals for a Soil Framework Directive (COM(2006) 232) and an Impact Assessment (SEC (2006) 1165 and SEC(2006) 620). A major element of the Strategy is that it has formulated requirements to prevent soil contamination, compile an inventory of contaminated sites and remediate those sites listed on the inventory. In line with the approach followed in SSEO, the working group on contamination focused on a whole-system approach – therefore starting from soil as compartment, rather than solely from toxic compounds as threats. The task group on contaminated land management concluded that: “*The management of contaminated land must follow the concept of Risk Based Land Management … applied on a case–by-case approach. When a problem of new soil contamination is found, immediate action is required*”; these ideas are in line with current Dutch regulations. Furthermore, as a recommendation, the long-term goal of soil management is formulated as sustainable land use and protection of natural resources. Considering the results of the SSEO programme, it can be stated that the EU-promoted concept of Risk-Based Land Management can be further underpinned by the methods that have been applied in the SSEO programme. Many of the research methods provide a better insight into local risks of mixture of contaminants, and they may thus be implemented as tools under the Soil Strategy, although formal adoption by the EU is still awaited.

33.4 Possible implications for national regulations: species, nature and area policies

Nature policies can be divided into policies directed at the protection of habitats and biodiversity in designated areas (Nature Conservancy Act, Ecological Main Structure, Birds- and Habitats Directive and its Natura 2000-sites) and policies directed at the protection of species (Birds and Habitats Directive, Flora and Fauna Act, Species Protection Plans, Red lists (national), Bird directive, Red lists (international)). The policies can be further divided into national (Nature Conservancy Act, Ecological Main Structure, Flora and Fauna Act, Species Protection Plans, Red lists (national)) and international (Habitat Directive, Natura 2000, Bird Directive, (inter)national Red lists) policies. The policies have a legal status, except for some of the national policies (e.g. Ecological Main Structure, national Red lists). The target of these policies is protection of general biodiversity, habitats, habitats of specific species, and of species (individuals of the species and their populations). All these policies aim at maintaining a favourable conservation status of their objectives. They usually try to achieve this by habitat protection (except in the Flora and Fauna act). The international policies state that no activities are acceptable which result in significant negative impact on the habitats or species. Pollutants may be one of the factors threatening the favourable status. Only in the Habitat Directive is this aspect specifically mentioned.

The studies in the SSEO programme have shown that the risks or effects of toxicant mixtures in field conditions cannot be interpreted straightforwardly from small sets of field observations and/or simple data analyses. The actual risk and local effects are often very dependent on the other stress factors (environmental conditions), which calls for location-specific assessment of risk. As shown in Chapter 13, the Target Values for single pollutants are not exceeded, whereas an effect of the mixture of pollutants is measurable (a drop in growth rate of up to 23%).

Given the scarce number of studies that show clear effects of toxicants on species and habitats under conservation, toxic stress has not been appreciated as one of the most important stress factors in Dutch nature conservation. The SSEO programme has shown that if toxic stress works in concert with other environmental stress factors, it may have a large impact. This calls for an increased awareness of the possible detrimental role of toxic stress on the conservation of species.

As a preventive policy, the factor of toxic stress should play a role in the assessments of the quality of areas for nature conservation (e.g. Natura 2000 sites). In policies directed at protection of specific species (Birds and Habitats Directive, Flora and Fauna Act, Species Protection Plans, Red lists (national), Bird directive, Red lists (international)), awareness of possible effects of toxic stress and mitigation of these factors may improve the protection of the species covered by these policies.

33.5 Regulations: needs and recommendations

Cases of existing, diffuse contamination are addressed in various national and international regulations. The instruments used for evaluating those cases are often directly adopted modifications of techniques that were originally developed for the regulation of chemicals. This concerns generic risk assessments, formatted according to the primary goal of the regulation: prevention of adverse effects of chemicals and their mixtures. Although this implies consistency, the lack of predictability of effects from quality criteria (as found in the SSEO programme and other studies) suggests that a novel approach needs to be derived to improve the management of diffusely contaminated sites. Based on specific regulations, we propose to implement a tiered system. A tiered system implies conservatism and simple, generic approaches in the lower tier, and site-specific approaches that more accurately predict impact magnitudes in higher tiers. Conceptually, a tiered system can link regulations as diverse as preventive chemical regulations, pesticide regulations and site-specific assessments of sanitation urgency. The consistency is in the common use of the risk assessment paradigm, and in the deliberate choice of conservative methods in lower tiers. In this way, a clear decision pathway can be envisaged (Figure 41), to be applied from prevention, via optimization of site management, to curation. For site management and curation, the problem definition not only considers ranking (e.g., the highest impact sites being sanitized first), but also future perspectives for those sites: “What will happen if we choose method *x* as a management option?”. The site-specificity in risk assessment, and the need to look at development perspectives (e.g., regarding land-use options in the future) both require ecological approaches in addition to ecotoxicological approaches. The ecotoxicological approaches can show when risks are too high – indicating the restrictions imposed by site contamination – while the ecological approaches could show prospects – indicating whether ecological criteria (like Good Ecological Status) can eventually be met. The toolbox for risk assessment needs extension with a toolbox for site management, whereby the latter can be of help to reach optimum ecological quality. We recommend the adoption of the tiering principle to link an array of national and international regulations, and – similar to chemical regulation – develop *Guidance* on using site-specific assessments in the context of all types of regulations. This is essention for site managers (next su-section) to solve their practical problems.

33.6 Possible implications for management of diffusely contaminated sites

33.6.1 General issues in site management: current effects

Although the evaluation of current effects is a regulatory issue, it has become clear that the actual effects that have been observed in the three research areas are not of such a magnitude that they would trigger immediate management action or sanitation. The managers of these sites apparently need not take specific, immediate action when the site use remains the same. They might consider scenario analyses (see below) in case site use changes. This conclusion needs not hold universally for other diffusely contaminated sites, where the type of contamination can be different, and where it has been present for a shorter time (reduced ageing, reduced adaptation), where the matrix has other sorption characteristics and where the receptors have different sensitivities. Effects will generally be higher with higher total concentrations, more complex mixtures, lower sorption and lower degradation rates (aspect of time) and more sensitive species, and will also be considered higher at geographically larger scales (aspect of space).

33.6.2 General options for site management: scenario analyses

The SSEO study areas were chosen due to the presence of toxicant exposure gradients and regulatory relevance; for all three sites, the management of contaminant risks is only part of a complex management situation. In two areas, large-scale plans are being developed to change the area considerably, such as the creation of secondary river arms in the Afferdensche and Deestsche Waarden (river floodplains), to reduce the probability of floods, the opening of the Haringvliet locks (in 2008) to increase tidal influences and the current practice of Staatsbosbeheer of creating wetland areas by deliberately letting water flood into polders in the Biesbosch. For the “toemaakdek” area in De Ronde Venen, site managers are keen to take risk reduction measures, triggered by the exceedances of the generic soil quality criteria. The question is: what can be learnt from the scientific studies done in each of the areas for the management of those areas? Note that this question was not the scientific target of the research; hence the remarks below are generalized options to improve site management.

In general, the generic risk assessment that was performed per compound to set the generic quality criteria in the past has followed the risk assessment paradigm (Figure 43). Site-specific risk assessment follows the same paradigm, although with site-specific information. First, the risk assessment problem formulation is specified as to the location and biotic responses of interest (top box). Thereafter, exposure assessments are made for the location under study (left branch), and an effects assessment is made for the biota being exposed (right branch). Extrapolation techniques are applied in these branches whenever necessary (see Solomon et al. (In press) for a comprehensive recent review). Finally, the risk estimation/characterization delivers the answers on risks as needed, resulting in risk management.
In the case of the SSEO research sites, we can thus provide a *first* risk characterization by applying the generic scenarios (that were used to derive the quality criteria), and these results are *status quo* assessment results in terms of quality criterion exceedance ratios per compound (risk quotients, calculated as local concentration divided by the criterion concentration, i.e. RQ = local concentration/criterion concentration, with RQ-values > 1 indicating potential risk).

Second, by using site-specific modelling (like with the models and approaches given in this report), we can provide risk characterizations based on *status quo* site-specific assessment scenarios, i.e. taking into account site-specific factors that influence exposure on the one hand, and those that influence the hazard assessment on the other. This means, for example, that the local soil conditions are considered, and that it is seen that the local bioavailability is lower than in the generic scenario. In addition, the criticisms of Risk Quotients are circumvented by more appropriate models. The execution of second-tier approaches would imply lower local risks (in the second tier) than expected from the risk quotient approaches. Also, it may mean that the local biota are considered rather than the generic species group that is considered in the first-tier hazard assessment. Again, lower or higher sensitivity than the generic species group implies lower or higher local risks than expected. Furthermore, ecological approaches are warranted in such cases, and could help to assess the possibility to reach a good ecological quality, given the local site characteristics and issues like refuge areas in the vicinity. From there, diffusely contaminated sites can be recolonized.

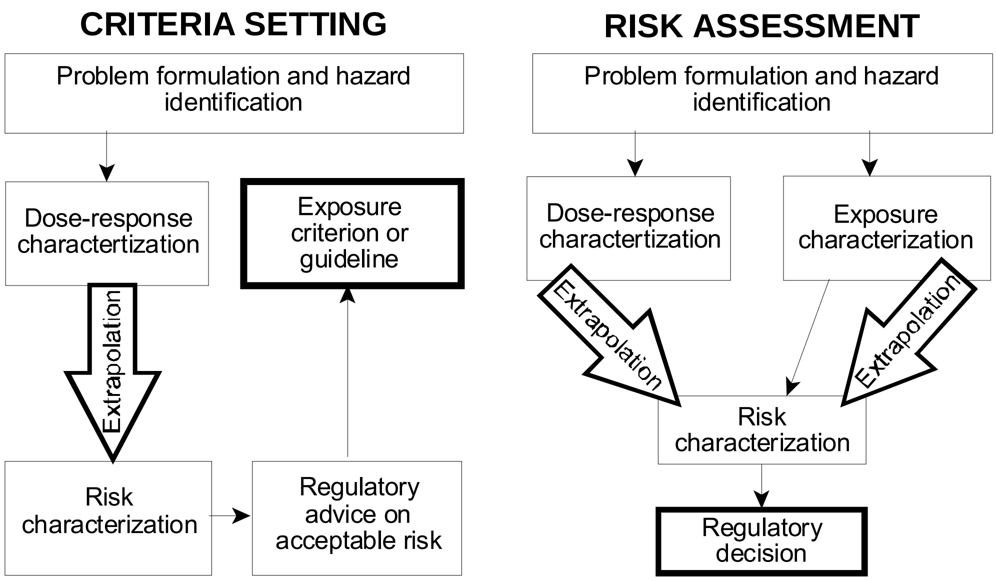

Figure 43. The risk assessment paradigm, presented for two purposes. (Left) Deriving a generic quality criterion. (Right) Deriving another regulatory decision from exposure and effect data. A risk assessment always considers a problem (e.g., setting a quality criterion or assessing the site-specific risks of a mixture), thereafter an exposure and a hazard assessment (left and right middle blocks), and finally an answer (the risk characterization). Validation is possible for two aspects: (a) predicted exposure levels in comparison to body residues found in field-collected biota, and (b) for risk characterizations (risk levels) in comparison to the magnitude of field responses. The latter is more complex than the former (see text).

Next to the *status quo* risk assessments in tier 1 (using generic criteria and yielding Risk Quotients) or tier 2 (site-specific risk assessments), it is possible *to run scenarios for different site or risk management options*. A scenario may be defined by a planned soil-use change, or any other general site management perspective. A scenario may also be defined as a consequence of tier-2 results indicating excessive risks. When a scenario is defined, this means that the risk assessment problem definition (see paradigm-figure) is refined again, but now not on *status quo*, but on a future, optional situation. For that scenario and problem definition, both the exposure and the hazard assessment need be done, to arrive at the scenario-specific risk characterization.

It is evident that the possible scenarios will vary between “no action – leave site as is” (when the site-specific risk levels are acceptable), via specific risk management, to sanitation (remove the contaminated substrate, which often includes removal and/or serious disturbance of the ecosystem). Looking at exposure, for example, risk reduction can be realized by immobilizing metals (when pH is kept high). Looking at risks, it should be realized that the set of species that will be present after a risk management action may be different (e.g., less sensitive) than the current set of species. In general, models will be needed to explore the consequences of the different scenarios.

Using scenarios implies acceptance of the novel/additional viewpoint for site management, whereby generic criteria are not sufficient for optimal planning. Site management is improved when there is an assessment framework that includes ecological criteria. Which management scenarios, when confronted with both ecotoxicological and ecological criteria, offer the largest improvement in ecological status? Using generic risk criteria only could lead to less improvement than dictated by the local ecosystem possibilities. The implementation of a broader approach (ecological options next to ecotoxilogical limitations) could be possible in relation to various current regulations for nature development, such as the Stimulation Plan Agricultural Nature management (SAN), the Survival Plan for Forests and Nature (OBN), and the Rural Areas Investment Plan (ILG). Formulating exactly *how* to implement the broader approach in these area-focused management plans is beyond the scope of this report.

33.6.3 Site management issues in SSEO study areas

33.6.3.1 Site management issues in the area of Ronde Venen

The area of De Ronde Venen is part of an extended area of so-called “toemaakdekken”. Large areas with peat in the western part of the Netherlands are covered with such a unique layer of topsoil. Subtle effects of metals in this layer and the terrestrial ecosystem were demonstrated in the SSEO programme. Removal of “toemaakdek” is not an option for remediation, considering its long history, the dimensions of the area (surface area and soil volume), and societal aspects. Besides, it was not shown that the level of effects should trigger remediation actions, according to the currently operational site-specific remediation thresholds in current soil policy, except for exceedance of Intervention Values for some metals. However, soil management will take place now and in the future. In an environmentally sound soil management of the “toemaakdek” regions, the observations made by SSEO and others can be taken into account. Soil management will affect the occurrence of adverse effects of metals on the terrestrial ecosystem in a positive or negative way. For instance, hydrological conditions are expected to have a large effect on the oxidation of peat, and subsequently on the mobilization of metals. The expectation is that a high water table will reduce the effects of metals in peat areas. Furthermore, depending on the type of nature development, the ecosystem will be more sensitive or less sensitive (based on its intrinsic characteristics and species composition), and thus more affected or less affected by metals. Key species and nature target types may have a different sensitivity towards metals.

33.6.3.2 Site management issues in the area of the Afferdensche and Deestsche Waarden

In soils of the Afferdensche and Deestsche Waarden along the Waal river, levels of cadmium and copper are moderate and those for zinc are still relatively high. In this floodplain area, flooding has a dramatic impact on the occurring species; flooding actually drives the population dynamics of small mammals and earthworms. Obviously, the “noise” induced by flooding largely overshadows effect “signals” of the above heavy metals. This implies that if we want to disentangle the signal from the noise, we should increase the number of replicates in a study. This has been done by adding data from the two floodplain sites of the SSEO programme (ADW and Biesbosch) to data of sites along the Scheldt river. In that study, clear effects of heavy metals on earthworm biomass and density were visible.

The Afferdensche & Deestsche Waarden will be included in major river rehabilitation plans of the Dutch government. In those plans, the floodplain will change due to the construction of a secondary channel and the removal of part of its minor embankment. This rehabilitation plan will induce changes in flow patterns and velocities during floodplain inundations, which have a direct adverse effect on the site-specific viability of species such as earthworms (Klok et al. 2007). Subsequently, changes in flooding may result in different patterns and amounts of sediment deposition, which are often contaminated, and contamination levels may even rise slightly. Not only freshly deposited sediments contain contaminants, stored deeper-layer floodplains sediments may also be heavily contaminated with heavy metals and other substances. The contamination degree of these polluted soils generally peaks in the soil layer at 0.5 to 2 m below ground level. Lowering a floodplain by taking away top layers of clay soil and exposing sandy layers with a lower binding capacity for compounds may result in an increased availability of pollutants for earthworms, which may have consequences for both earthworms and earthworm predators like the badger *Meles meles* and the little owl *Athene noctua vidalli* . These are species that have protected status in the Netherlands. For these species, floodplain rehabilitation plans may greatly reduce the amount of high quality foraging habitat as a result of low availability of their food (earthworms). On top of that, these predators may suffer from secondary poisoning, given the fact that surviving earthworms may contain higher pollutant levels.

33.6.3.3 Site management issues in the area of the Biesbosch

In the area of the Biesbosch, soil metal concentrations are especially high, with levels of zinc and cadmium exceeding the Dutch Intervention Values at all or most sites sampled within the SSEO programme. Considering the scale of pollution and the local situation, soil cleanup or removal of the pollution is not an option. Metal availability in the Biesbosch soils, however, has been shown to be rather low due to favourable conditions of high soil organic matter and clay content and high soil pH. As a consequence, relatively low metal concentrations were measured in several organisms, such as plants and arthropods. Nevertheless, some organisms, like snails and earthworms, did show increased metal concentrations, rendering them potentially at risk. Also for small mammals, metals were shown to be the most relevant pollutants, followed by PCBs (at frequently flooded sites). Especially carnivorous mammals appeared to be at risk due to metal pollution. Risk mitigation strategies should aim at keeping metal availability as low as possible, e.g. by maintaining the high soil pH conditions. The plans to increase tidal influence by opening the Haringvliet sluices may help to realize this aim. The creation of wetlands by flooding polder areas may also reduce metal availability by increasing the probability of forming metal-sulphide complexes not being available for uptake by organisms. However, monitoring seems advisable to ensure that metal availability remains low, as soils have the tendency to show natural acidification under the influence of microbial activity.

33.7 Managing diffusely contaminated sites: needs and recommendations

When a toolbox for risk assessment is made according to a tiered system, and when ecological criteria are also embedded in the assessment better than before, it becomes feasible for site managers to optimize the ecological quality of their sites. When they apply current methods, they often need to apply a strict interpretation of the regulatory risk limits per compound, and this may lead to management practices that are neither ecologically effective nor cost effective. Site mangers need guidance on all aspects of site management, which includes not only risk assessment (defining what is not feasible for a diffusely contaminated site), but also ecology-based approaches to help them reach best ecological status, and methodologies to take the broader perspectives into account (e.g., cost-effectiveness, spatial aspects). It is advisable to develop such methodologies. In addition to defining the problem (ecological risks of diffuse contamination), such approaches may help to find solutions, and help to reach optimal ecological quality.

34 Conclusions from integrating SSEO

By: L. Posthuma, M.G. Vijver & H.J.P. Eijsackers

Scientific findings – with linkage to regulatory issues

1. Diffuse contamination is present at the three research sites of SSEO. Inventories within the Netherlands have also shown a widespread occurrence of contaminated areas, often with vast volumes of diffuse contaminated soil, sediment or water. Diffuse contamination is present when the regulatory Target Value is exceeded. The origins of the existing diffuse contaminations are diverse, and originate from past land use, current intensive agricultural use and contamination of nature areas in the vicinity of industrial sites. In the research areas, there were also cases where the Intervention Value was exceeded, identifying cases with “serious contamination”. National inventories have also shown large numbers of sites with such exceedances.
   → From the viewpoint of national and international chemical regulation, this indicates that “non-clean” environmental compartments exist in large numbers, volumes and areas. Exceedances up to “cases of serious contamination” also occur frequently. This implies the need for policy approaches to handle those sites.
   → From the viewpoint of responsible authorities for diffusely contaminated sites, there is a clear regulatory and societal trigger to investigate the implications of diffuse contamination for ecosystems. In societal terms, exceedances of quality criteria are often implicitly interpreted in terms of the presence of unacceptable adverse effects, while the exceedances conceptually imply the presence of unacceptable risks (increased probability of unacceptable effects). So far, there are no (or few) adopted approaches to assist site managers, such as the Dutch Sanitation Urgency System[16].

2. Effects of diffuse and serious contamination in the environment were studied in the SSEO research programme. This research has demonstrated a wide range of ecological responses, from little or no effect at high exposures, to large effects on valued species or ecosystem characteristics at low exposures. The wide variability is attributed to three factors (each with large variation between sites):
   a. The local mixture of contaminants (composition, concentrations)
   b. The local site characteristics that influence exposure through different sorption characteristics
   c. The locally-exposed biota, with varying sensitivities.

   Observing effects in diffusely exposed systems may be obscured due to the effects of other stress factors in the natural systems.
   → From the perspective of chemical regulations and regulations on compartments (water, sediment, soil), and as seen from the view of species and ecosystem protection, diffuse pollution is a complex problem, since those regulations have both good chemical status as well as Good Ecological Status (or similar concepts, like vital populations of non-target species) as final endpoints.
   → For authorities responsible for diffusely contaminated sites, diffuse pollution remains a complex problem, since they may use environmental quality criteria to classify the sites as clean, slightly or seriously contaminated (“clean” is defined by concentrations of toxic compounds lower than the Target Value; diffuse contamination is found when the

[16] SUS: VROM, Van Hall Instituut. 2000. Sanerings Urgentie Systematiek Versie 2.2. Version 2.2. Den Haag/Groningen en Leeuwarden: VROM/Van Hall Instituut.

concentration ranges between the Target Value and the Intervention Value; and serious contamination is defined by concentrations exceeding the Intervention Value), but there are only few or no accepted methods for local risk management in the case of diffuse pollution.

3. Considering the diversity of effects and the underlying causes, the SSEO programme has not resulted in additional evidence to suggest a general change in the processes whereby generic risk assessment is used to derive environmental quality criteria and applies prevention principles to avoid the release of dangerous novel compounds on the market. In contrast, the diversity of effects suggests that using exceedances of quality criteria to guide risk management is not advisable as a sole assessment in cases of diffuse pollution. Such exceedances still offer no insight into the kind and magnitude of ecological responses. Generic risk limits are just not equivalent to quantitative effects, due to the three major factors mentioned above.
   → From the viewpoint of chemical regulation, changes in generic risk assessment could systematically yield more stringent or less stringent criteria. In case of change, another validation to reach the same status of regulatory acceptance as the current, generic approach (validated in the sense that they most often provide sufficient protection) is mandatory. This will take some time, because before such novel methods may be adopted, it must be shown that they improve the commonly used evaluation technique.
   → From the viewpoint of responsible site authorities, the use of quality criteria does not sufficiently solve their risk management problem of ecosystems already contaminated, since the clean-up of sites that are exposed at levels higher than Target Values (or Intervention Values) is not realistic due to their huge numbers and vast volumes. Other methods are needed to support the authorities responsible for the management of contaminated sites to match site use to the local contamination. Site-managers are confronted with questions in which environmental integrity, cost-effectiveness of measures and an integrated view of realistic management options are to be considered.
   → When considering both types of regulation (chemical-oriented versus site- or species/ecosystem-oriented), a possible misfit between approaches might occur, whereby the chemical-oriented policies are interpreted as predictors of effects, whereas the site-oriented approaches do not always substantiate those expected effects. However, the misfit is artefactual. The quality criteria were specifically designed for a limited use (i.e., prevention and detection of unacceptable risks), and it has always been implicitly accepted that further investigations are necessary to describe the ecological consequences of exceedances. Thus, efforts are needed to embed the concept of tiering in any set of regulatory frameworks.

4. The SSEO programme has used a multitude of methods to investigate local exposure levels and the occurrence (and magnitude) of local effects of diffuse contamination. The methods consist of measurement techniques, modelling approaches, or combinations of both. In some cases, a formalized triad approach has been used. This approach is based on the concept of Weight of Evidence, by weighting observations from different Lines of Evidence (e.g., modelling, bioassays, field inventories). The methods can be brought together in a site-oriented approach in risk assessment. They can be considered candidate methods to be applied in a tiered system of risk assessment, whereby the first tier consists of evaluation using generic quality criteria (screening-level risk assessment to discriminate sites), and the higher-tier approaches consist of refined, site-specific risk assessments. All tiers universally apply the risk assessment paradigm (basic consistency between tiers), but only the refined assessment considers more site-specific information in the exposure and effects assessment steps. This yields more refined information on the local risks. Often, according to general principles for

tiered systems, the results of refined assessments are less conservative than those of screening-level assessments.

→ Again from the viewpoint of national and international chemical regulation, the more widespread adoption of a tiered system is a logical extension of earlier adoptions of this principle. Two existing examples are the evaluation of new pesticides in the European Union and the sanitation urgency system applied for ranking the priority of site remediation in the Netherlands (see previous footnote). A higher-tier method would be required especially for sites with existing, diffuse contamination. A tiered system matches recently adopted national and international regulations (like the EU Water Framework Directive); a conceptual linkage is therefore needed between evaluations based on quality criteria (good chemical status) and evaluations based on biological quality (Good Ecological Status). As a result, there will be three regulatory approaches: prevention, risk management in cases of diffuse pollution, and curation in cases of serious pollution. Evidently, novel cases of pollution remain to be regulated according to current policies, and this generally leads to the obligation of site owners to clean the site.

→ From the viewpoint of responsible authorities for diffusely contaminated sites, a tiered system should not only address the site contamination itself (as based on the ecotoxicological implications of contamination) and the *limitations of use* implied by that contamination, but also scenarios for *optimized site management*. The latter should result in the best ecological status that can be reached in the local context. This implies that practical methods are needed to assess not only the current ecological status (like the Good Ecological Status concept) and risks with current site use, but also the ecological perspectives for the development and use of contaminated sites in case changes of site use are planned. Scenario analyses could have a great potential for this purpose, both in terms of limitations (by local toxicants) and ecological potentials (according to the local context).

5. Similar to the generic risk assessment (tier-1 methods), higher-tier methods to assess the implications of diffuse contamination can be scientifically underpinned (e.g., by the methods applied in the SSEO programme). Likewise, sets of scientific approaches can be formally adopted for practical problems (*Guidance on site-specific risk assessment of existing contamination*). To facilitate the use of such methods, a scientifically underpinned toolbox for site-specific risk assessment[17] can be envisaged, in which models and measurement approaches are made available for broader, practical use (e.g., to make a site-specific evaluation of cases of diffuse contamination). Models and measurement techniques should be formulated so their use limitations are known. Neither models nor measurement techniques should be applied beyond their limits of use. In SSEO, various models and approaches were the subject of a validation study, and this resulted in improvements of some models. All models and methods considered can be included in the toolbox, whereby it should be acknowledged that some models over-predict exposure and effects. As a whole, the set of validation studies is, however, limited, but many models have a proven legacy of providing useful insights. Therefore, some have reached the status of "validation by adoption in practice".

   → From the viewpoint of national and international chemical regulation, the generic models for exposure and effects assessment have been formally adopted, for example in Guidance Documents. It is likely that novel scientific insights (like those generated by the SSEO programme) will in practice lead to a continuous evolution of the generic evaluation methods, and regular revision of the quality criteria. Revision of the criteria has major implications. On

[17] The Dutch government has adopted such a toolbox in their new soil policies. See: RIVM, RIZA, Alterra. 2007. Risicotoolboxbodem. Bilthoven: RIVM.

the one hand, governments generally prefer to be consistent, and "old" criteria will be replaced by "new" criteria only when it is proven that they are better. On the other hand (when chaned criteria are adopted), the number of sites considered to be diffusely contaminated will change.
→ From the viewpoint of responsible authorities for diffusely contaminated sites, a toolbox for site-specific risk assessment (or other practical methods) can be of great practical value, provided that the generic risk assessment is always (or at least in most cases) a relatively conservative "signal" for using it. Such a toolbox should provide better insight into local exposure and the type and magnitude of local effects. These scientific outputs require a regulatory framework for interpretation. Similar to setting quality criteria, there is an aspect of regulatory value judgment, in this case on the acceptability of local risks and effects, in comparison to the effects of other stressors and the context of the situation.
→ From the viewpoint of linking chemical-oriented regulations to the other regulations, the toolbox for risk assessment can help to merge classically separate scientific and regulatory fields, thereby supporting a clear regulatory need for more integrated assessments and the requirement that has evolved[18] to execute cost-benefit analyses.

Major regulatory issues

1. Toxic compounds are addressed in various adopted regulations, that vary from preventive chemical-oriented policies and site and compartment-oriented policies, to species- and ecosystem-oriented policies. In recently-adopted regulations, the classical approach, where the environmental compartments must have a chemical quality which meets regulatory criteria, there is an argument that has gained considerably in importance: Good Ecological Status.
2. Given the increased importance of Good Ecological Status, there is an urgent need to establish appropriate linkages between the regulatory domains of chemical compounds, and site- and system-oriented approaches. Ecological quality is (and will always remain) the only universal currency which allows the avoidance of any "apples-and-oranges problem": an increased ecological weight in a tiered system should be considered to solve site problems with diffuse pollution. Ecology-based tiered systems must be embedded in the frameworks of all regulations alike. If not, national policy decision-makers and site managers shall be confronted with conflicting results.

Status of the report

1. This report concerns a first attempt to integrate most of the data from the SSEO programme, based on the studies of SSEO researchers and several associated research lines, mostly for three research areas.
2. A formal scientific review of the effects of diffuse pollution in ecosystems reported in the open literature may reveal additional information and further quantitative insights.

[18] EZ/VROM/Justitie. 2003. Effectbeoordeling voorgenomen regelgeving. Ministeries van EZ, VROM en Justitie. Report nr 03ME19.

Acknowledgements

The studies for this report were supported by the Research Council for Earth and Life Sciences (ALW) of the Netherlands Organisation for Scientific Research (NWO), under NWO project number 014.23.094 and NWO project number 014.23.095, which was in turn funded by the Ministries of VROM, LNV, V&W and OC&W. The integration work of RIVM personnel involved in this project was further co-funded from the common programme budgets from the Ministry of Housing, Spatial Planning and the Environment (VROM), and by the Director-General of RIVM, which provided co-funding from the Strategic Research Spearhead on Quantitative Risk Assessment for ecosystems (QRA) under project number S/860703 (QERAS). The project team acknowledges the valuable contributions of data formats, explanations, data analyses, written contributions and stimulating discussions from many researchers, research leaders and site coordinators who functioned under the auspices of the SSEO research programme. Without those contributions, there would be no data or other inputs, and thus no overview report.

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