POLYBROMINATED FLAME RETARDANTS

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Since 1998 brominated flame retardants (BFRs) have drawn a lot of attention at the Dioxin conferences. Although they have been reported to be present in the environment in the early 1980's, the discovery of their presence in human milk and in whales, both presented at Dioxin 1998 in Stockholm, initiated much activity in environmental laboratories. Many interesting papers have been presented during the Dioxin conferences in Venice, Monterey and Gyongju. Many laboratories have developed analytical methods, in particular for polybrominated diphenylethers (PBDEs). PBDEs have been detected in various biota and sediments from the aquatic environment, air, human milk, blood and adipose tissue, bird eggs, various foodstuffs and other matrices.

A substantial number of papers of the total of 24 papers presented at Dioxin 2002 gives new information on human exposure to BFRs. Van Bavel *et al.* analysed 220 blood samples from Sweden. Background levels varied between 1 and 6 ng/g for the sum of PBDEs, on a lipid weight basis (lw.), but in 5 % of the samples SPBDE concentrations of over 30 ng/g lw. were found, possibly indicating an unknown source of PBDEs. Thuresson *et al.* found decaBDE concentrations of up to 300 ng/g lw. in blood of rubber workers, producing and handling rubber containing decaBDE as a flame retardant.

Indications of a relatively short half-live of decaBDE in human blood (ca. 15 days) were found. TBBP-A concentrations in blood from workers in a printed circuit board recycling plant were below the limits of quantification, probably due to a limited exposure and short half-live of TBBP-A in humans. Choi *et al.* report a 44-fold increase of SPBDE concentrations in human adipose tissue from Japan, over the period 1970-2000, resulting in SPBDE concentrations of 466-2750 pg/g lw. The presence of brominated dioxins and furans in human adipose tissue is reported for the first time. No significant trend was found from 1970 to 2000, 5.1-4.0 pg/g respectively for SPBDD/Fs. The PBDE data are confirmed by Hori *et al.* who report an increase of PBDE concentrations in stored human milk samples of ND to 1.6 ng/g lw, from 1973 to 1988 and levels between 1 and 2 ng/g since then. The presence of PBDEs in human adipose tissue from the Czech Republic is also reported (Crhova *et al.*) S.-J. Lee et al. report human blood levels of PBDEs in Korea. Ryan *et al.* report 10-fold higher SPBDE concentrations in Canadian human milk, compared to those in Swedish human milk, earlier reported by Meironyté ad Norén. Also, a 15-fold increase is reported for SPBDE levels in human milk from Vancouver. Time trends of PBDEs in human milk from Uppsala, Sweden (Darnerud *et al.*) confirm the earlier work of Meironyté and Norén. Lind *et al.* give an overview of the intake of PBDEs and HBCD. Interestingly, the estimated HBCD intake, although based on somewhat preliminary data, is 3-fold higher than that of SPBDEs. The most important food group for PBDEs intake is fish, which constitutes two thirds of the total intake. Petreas et al. report high PBDE concentrations (up to 500 mg./g lw. for BDE47) in human serum from California, as well as relatively high PBDE concentrations in human adipose tissue.

The quantity of new information on human exposure to BFRs, in some cases resulting in relatively high PBDE concentrations in human blood, milk, and adipose tissue, further emphasizes the need for more toxicological information on BFRs. Such information is badly needed for a better understanding of the relevance of the concentrations reported and their possible effects. Unfortunately, almost no new

toxicity data have been forwarded for presentation at this session. Further toxicological studies should therefore be strongly encouraged. Huwe *et al.* present the results of a mass balance feeding study of a commercial octabromodiphenyl ether mixture in rats. The bioaccumulation decreased with higher bromination. Stapleton *et al.* suggest a possible degradation of BDE183 down to BDE154 in a feeding study of common carp, either by biotransformation or by debromination prior to the uptake by the carp.

Knoth *et al.* report on a different source of PBDEs: house dust. Relatively high concentrations of in particular BDE209 (mean 1394 ng/g, maximum 19,100 ng/g) were found. Leakage of BFRs from consumer products is suggested, but is not confirmed. Exposure paths of PBDEs are not clear yet. It is unclear if for PBDEs the same exposure routes are valid as for other persistent organic contaminants: are the food intake processes predominant or is there any possibility of another process through inhalation or through dermal contact? These questions would require more attention in the near future.

In addition to papers on human exposure, a number of other papers focus on BFR levels in environmental samples. Sakai *et al.* report on PBDE concentrations in sediment cores from Osaka Bay, Japan. The PBDE peak was found in the upper levels of the cores at a point where PCB concentrations had already substantially decreased. This trend in the sediment cores was related to the industrial production of PBDEs. Relatively low SPBDE concentrations (0.4-18 ng/g dry weight) were found in Portuguese river sediments (Lacorte *et al.*). Higher PBDEs levels were found in sediment from major Minnesota rivers (USA) (Oliaei *et al.*). In contrast, SPBDE concentrations in South Korean sediments are low (1.7-5.9 ng/g dry weight), but TOC data are needed for a correct interpretation. Ohta *et al.* detected PBDDs/DFs and PXDDs/DFs in marine sediments. TBDD, PeBDD and TXDD of 2,3,7,8 substitution congeners in particular were relatively high in concentration. They suggested that PXDDs/DFs TEQ values could exceed the TEQ of PCDDs/DFs.

Lepom *et al.* report the presence of BDE209 in bream from the river Elbe (Germany), in concentrations up to 37 ng/g lw. This is the first report on the presence of decaBDE in European freshwater fish. DecaBDE was not found in eel from the same river.

R.G. Lee *et al.* report atmospheric concentrations of PBDE in samples from the Irish west coast and the UK. The Irish samples show 3-fold lower PBDE concentrations than those from the UK.

The results of the second international interlaboratory study on BFRs are reported by de Boer *et al.* Results were obtained of 36 laboratories from 13 different countries. Eighteen laboratories had participated in the first round. This shows the increasing interest in this field over the last three years. Progress was made for the comparability of BDE99 and other PBDE data, but the analysis of BDE209 in biota and sediments is still not under control by the majority of the laboratories. A coefficient of variation (CV) of 65 % (n = 13) was obtained for the analysis of a sediment sample. The CVs for the biota analyses were >100 %. Obviously, laboratories should be encouraged to improve their BDE209 analysis. Relatively few sets of results were returned for HBCD and TBBP-A, which shows that many laboratories are still installing methods for these BFRs.

Hori *et al.* and Eljarrat *et al* report two new methods for PBDE analysis.

A paper of Muir and Alaee on a cost/benefit analysis of BFRs concludes that a continued use of BFRs would lead to very large human heath and economic costs and consequences.

The data presented show that PBDEs are global contaminants that have a tendency to increase in concentration in the environment and human samples in some parts of the world, in particular in North America. The exposure data call for further toxicological studies to assess their relevance and possible effects. The analytical comparability is acceptable for a number of PBDE congeners but not for BDE209. It should, therefore, be kept in mind that some of the BDE209 data reported do have the quality that is required for publication. Laboratories are encouraged to improve their methods for this BDE, and further develop methods for other BFRs such as HBCD and TBBP-A.

BIOANALYSIS

Detection and quantitation of polychlorinated dibenzo-p-dioxins (PCDDs), biphenyls (PCBs) and dibenzofurans (PCDFs) and related chemicals (polycyclic aromatic hydrocarbons, chlorinated pesticides, brominated flame retardants, and other POPs) has commonly employed instrumental analysis procedures (i.e. high resolution gas chromatography and mass spectrometry (HRGC/MS)) that require highly sophisticated equipment and training and are also very costly and time-consuming. Over the past several years, numerous bioanalytical and bioassay methods have been developed that can provide an inexpensive and rapid alternatives in which to detect and/or estimate the relative biological/ toxic potency of individual POPs or complex mixtures containing POPs. Bioanalytical methods are generally based on the ability of a chemical(s) to be specifically recognized and bound by antibodies (immunoassays) or their ability to induce a specific biological response in vitro or in cells in culture (bioassays). Immunoassay-based bioanalytical approaches utilize reagents (antibodies) which are generated against a specific chemical structure and as such, they tend to be relatively specific for a given chemical or closely related chemicals. Bioassay approaches for chemical detection are generally less specific than immunoassay approaches and they are based on the ability of a chemical or chemical class to affect a specific biological response or pathway. Accordingly, taking advantage of the molecular mechanism of action of PCDDs and related chemicals, several Ah receptor (AhR) based bioassay systems have been developed and optimized for the detection and quantitation of PCDDs, PCDFs, PCBs and related POPs. Numerous studies have demonstrated that bioanalytical methods can be used as valid approaches for the rapid and inexpensive detection and relative quantitation of PCDD and related chemicals and even the identification of novel POPs. However, even given the advantages of bioanalytical approaches, these methods have not gained widespread use and this likely results from the lack of regulatory acceptance of these new methods. Although these bioanalytical methods have been in place for years, extensive validation studies are now being conducted and as such, these methods are only now being examined in detail and considered as screening methods. The availability of fully-validated and accepted alternative bioanalytical methods for POPs detection and analysis will greatly facilitate many large scale screening studies where the equipment and/or funding is limited (as it usually is). The results and process necessary to gain regulatory acceptance of these alternative bioanalytical methods are critical issues that need to be resolved.

This session on Bioanalysis includes nine platform presentations that address the development, application, validation and limitations of selected bioanalytical approaches. The first talk by Dr. Akira Okuyama describes the development of an enzyme-linked immunosorbent assay (ELISA) for quantitation of coplanar PCBs using a monoclonal antibody generated against PCB118. These studies are unique in that the majority of available ELISA assays are for the detection of PCDDs. The assay was sensitive, with a minimal detection limit for their ELISA assay was 34.6 ng/ml (1.7 ng/well) for PCB118. Although this antibody shows minimal cross reactivity with numerous other coplanar PCBs (i.e. 3,3',4,4',5-TCB) and essentially no cross-reactivity with PAHs and related compounds, given the ubiquitous nature of PCB118, the ELISA can be used as a sensitive tool for prescreening of samples for coplanar PCBs. The next two talks describe the development and application of new technologies for detection of PCDDs and PCDFs. Application of the AH-immunoassay (AH-I) for dioxin screening is presented by Dr. Yasuo Kobayashi. This assay systems measures chemical-dependent activation of the DNA binding activity of the AhR complex and measurement of the amount of complex using an antibody-based colorimetric assay. Using this system, the authors report that this bioassay has a minimal detection limit of 1 pg TEQ/g sample and a dynamic range of measurment of~20,000 pg

TEQs/g. Significant cross-reactivity with other HAHs and PAHs exists and the degree of cross reactivity correlates with WHO-TEFs. Analysis of ash and soil samples revealed a good correlation between TEQs estimated by the AH-I and HRGC/MS, although a 10-13 fold higher estimate was obtained with the AH-I system. Several explanations are put forward to account for this elevated activity. The second new bioassay is the real-time PCR assay (AhRC PCR) and is presented by Dr. Randy Allen. This bioassay assesses chemical-dependent activation of the DNA binding activity of the AhR complex by a given sample and estimates the amount of AhR ligand by quantitation of the amount of DNA that is captured by the activated AhR using the polymerase chain reaction and fluorescent probes. Increasing amount of AhR ligand in a given sample (i.e. PCDDs, PCDfs and PCBs) will result in an increase in the amount of DNA captured, with a resulting increase in fluorescence. The authors report that the minimal detection limit for TCDD was than 0.31 pg per well.

The next two talks describe application of cell-based gene expression bioassay systems for the identification of bioactive chemicals present in complex mixtures. Dr. Helena Olsman describes the application of the ethyoxyresorufin O-deetylase (EROD) cell bioassay for bioassay-directed chemical fractionation procedures to identify chemicals present in anaerobically digested organic matter that exhibit dioxin-like activity. The authors demonstrated that di- and polyaromatic fractions of both mesophilic and thermophilic treatments induced EROD activity. Since further fractionation resulted in higher overall inducibility, suggesting the presence of inhibitors in the complex mixtures, real world samples may actually contain much higher levels of TEQs than estimated from the original extracts. Dr. Corine Houtman describes the application of recombinant AhR- and estrogen receptor-response luciferase cell lines for use in toxicity identification evaluation (i.e. bioassay-directed chemical fractionation procedures) for detection of endocrine disrupting chemicals in sediment samples. Initial experiments established the methodology and its subsequent application to sediment samples allowed partial fractionation of polar and nonpolar chemicals with estrogenic activity. These studies suggest that these systems can be used to identify unknown bioactive chemicals in complex mixtures.

Although numerous laboratories have used the recombinant AhR -response luciferase cell lines for detection and relative quantitation of PCDDs and related chemicals and have shown that the results of these bioassay analysis are comparable to that of HRGC/MS, these results have not been considered by many as truly quantitative TEQ results. Dr. Ilse Van Overmeire discusses the required performance characteristics necessary to validate the recombinant luciferase cell bioassay system as a quantitative screening method. They describe an approach to determine the confirmation range of bioassay data necessary to reduce the number of true negative or true positive samples that must sent for confirmation by HRGC/MS. In addition, they describe a calculation model for the detection limit of a procedures. Although recovery determinations are required for quantitative instrumental analysis methods, these established procedures can not be used for bioassay screening methods. Dr. David Brown describes recovery determination approaches that have application for cell bioassay analysis. These corrections methods need to be included in bioassay analysis in order to avoid under reporting of the TEQ contamination of samples. The authors report that the use of biologically inactive congeners as internal spikes for their cell bioassay studies allow for accurate estimation of recovery efficiency.

The focus of the final two talks is related to the use of bioassay and bioanalytical approaches for bio-monitoring and screening of samples for further analysis. Dr. Hidetaka Takigami describes validation studies for biomonitoring of waste PCB in contaminated samples of mineral insulating oil and capacitor oil. Using a combination of the dioxin-response luciferase cell bioassay system and a PCB immunoassay with sample cleanup procedures, the authors not only demonstrate a good correlation between results obtained using each bioassay method, but their results support the use of these methods for biomonitoring purposes. In the final presentation, Dr. Kim Hooper and coworkers describes the results of studies designed to use the luciferase cell bioassay system to both screen

mothers in southern Kazakhstan to identify those containing high levels of TCDD and to map the time course of TCDD elimination via breast milk. Their results not only suggests that the luciferase cell bioassay is useful both as a screening assay to select individuals with high TCDD TEQs for epidemiology studies, but that the bioassay can also be used as a quantitative measure of TEQs in breast milk samples.