ANALYTICAL EXPERIANCES WITH THE GERMAN ENVIRONMENTAL SPECIMEN BANK: POLYBROMINATED DIPHENYLETHERS IN DEER LIVER SAMPLES AND CORRESPONDING SOILS

Olaf Paepke[1], Christa Schröter Kermani[2], Dieter Stegemann [3], Frank Neugebauer[1], Peter Ebsen[1]

[1] Eurofins GfA GmbH, Neuländer Kamp 1, 21079 Hamburg, Germany
[2] Federal Environment Agency, Corrensplatz 1, 14195 Berlin, Germany
[3] Eurofins GfA, P.O. Box 410128, D-48056 Münster, Germany

Introduction

The German Environmental Specimen Bank (ESB) is a monitoring instrument of the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. The ESB is managed by the Federal Environment Agency and operated by contracted research institutes and university groups with special competencies in the particular fields. After two decades of operation the ESB provides now a continuous historical record of the state of the environment in Germany in this period. It allows the retrospective monitoring of pollutants to identify temporal trends and spatial differences.
Due to growing concern of brominated flame retardant (BFR) findings in humans and in the environment we analyzed polybrominated diphenyl ethers (PBDEs) in human samples collected in the frame of the ESB[1]. After the EU banned technical Penta- and Octa- BDE in 2004, we continued the measurements for PBDEs by analyzing environmental samples like herring gull eggs [2,3], deer liver and soil samples.
This paper will focus on spatial differences for PBDEs in soil and in deer liver and on potential correlation of PBDEs found in deer liver and the corresponding environment as demonstrated by forest samples like humus and surface soil samples.
The same soil and deer samples were analyzed for dioxins (PCDDs/PCDFs) and dioxin-like PCBs [4])

Materials and methods

The ESB is founded on a high degree of continuity for all steps. All important working steps are regulated by specific standard operating procedures (SOPs). These SOPs describe all main operations in detail and can be accessed on the under ESB homepage[5]. Sampling of environmental specimens is performed annually or biannually in 13 (here only 9) ecologically representative areas which reflect the environmental situation in Germany.
Samples: In Table 1 the origin of samples and a short site description is given for all samples analyzed. The soil samples are represented by the humus/organic matter (the litter layer of plant residues in relatively undecomposed form) and the A-horizon-surface soil (layer of mineral soil with most organic matter accumulation and soil life). Soil samples were collected in 2002 and 2006. For deer liver we analyzed samples collected within three different sampling periods: 2001, mainly 2003 and mainly 2007.

Table 1: List of deer liver and soil samples for PBDE analysis (sampling site and year of collection)

Origin	Site description	Soil year of sampling		Deer liver year of sampling		
Solling	**Second highest and largest low mountain range in Northern Germany.**	**2002 Humus [a]) 2002 A-H [b])**	**2006 Humus 2006 A-H**	**2001**	**2003**	**2007**
Bornhöved	**Main watershed between the North- and Baltic Sea**	**- 2002 A-H**	**- 2006 A-H**		**2003**	**2007**
Pfälzerwald	**Germany's largest connected forest area in a range of low mountains**	**2002 Humus 2002 AH**	**2006 Humus 2006A-H**		**2003**	**2007**
Bayerischer Wald	**The Bavarian Forest National Park is Germany's first national park**	**2002 Humus 2002 A-H**	**2006 Humus 2006 A-H**		**2003**	**2007**

Harz	**The Harz National Park is Germany's largest forest national park.**	**2002 Humus 2002 A-H**	**2006 Humus 2006 A-H**		**2003**	**2007**
Berchtes-gaden	**The only high mountains national park in Germany and an area of the Limestone Alps with international relevance.**	**2002 Humus 2002 A-H**	**2006 Humus 2006 A-H**		**2002**	**2006**
Tertiärhügel-land	**The Upper Bavarian Tertiary Uplands are a part of the Southern German Molasse Basin.**	**2002 Humus 2002 A-H**	**2006 Humus 2006 A-H**		**2002**	**2006**
Warndt	**Near-urban area between the forest-industrial regions of Saarland and Lorraine.**	**2002 Humus 2002 A-H**	**2006 Humus 2006 A-H**	**2001**	**2005**	**2007**
Dübener Heide	**Region in the „chemical triangle" of Central Germany.**	**2002 Humus 2002 A-H**	**2006 Humus 2002 A-H**	**2001**	**2003**	**2007**

[a]) Humus/Organic matter: Litter layer of plant residues in relatively undecomposed form
[b]) A-Horizon/ Surface soil: Layer of mineral soil with most organic matter accumulation and soil life.

Analytical methods

All analyses were performed following the isotope dilution method. 13 native standards (BDE Nos. 17, 28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, were obtained from Cambridge Isotope Laboratories (CIL), Andover, USA, one native standard – BDE No. 209 – was from Wellington Laboratories, Guelph Canada, two were from Accu Standards – BDE Nos. 75, 6 internal C13 labelled standards - BDE Nos. 28, 47, 99, 153, 154, 183 and 209 - were delivered by Wellington, Canada, one – BDE 100 – was from CIL. Solvents were delivered by Merck (n-pentane), Promochem (cyclohexane, hexane, dichloro methane), Baker (diethyl ether), and Mallinckrodt (ethanol, toluene). Silica gel, alumina oxide, sodium sulphate and potassium oxalate and sulphuric acid were obtained from Merck.

Soil samples: A total of 10 g air dried sample was mixed with a mixture of 7 internal BDE standards and treated with toluene in a soxleth extractor for 10 hours. After extraction the toluene extract was treated with conc. sulphuric acid. After evaporation of toluene the remaining extract was dissolved in hexane.

Deer liver samples: A total of 15 – 20 g tissue was homogenized and mixed with sodium sulphate. Before column extraction a mixture of 7 internal BDE standards was added to the sample (100 pg / sample for each congener). For column extraction a mixture of cyclohexane and dichloro methane was applied. The extract was washed with water and dried over sodium sulphate. After solvent evaporation gravimetric lipid determination was performed.

Clean up of all soil and deer liver extracts was performed by acid treated and activated silica gel and alumina oxide column. The final extract was reduced in volume by a stream of nitrogen, the final volume was 50 µl containing C13 labelled BDE 139 as recovery standard. The measurements were performed using high-resolution gas chromatography/high resolution mass spectrometry (HRGC /HRMS, HP 5890 coupled with VG Autospec) at RP = 10,000 using a DB 5 (30 m, 0,25 mm ID, 0,1 µm film) column for gas chromatographic separation. The two most abundant masses were used for measurement (M+ for Tri- and Tetra-BDE, and (M–2Br)+ for Penta- to Hepta-BDE). The identification of BDEs was based on retention time and isotope ratio. The quantification was performed by using a five point calibration curve.

QC/QA measures: For reason of quality control a number of internal and external procedures are performed: For each batch of 6 to 10 samples a QC control sample and a blank sample were analysed in parallel. In addition, a number of samples was analyzed in duplicate. The laboratory participated successfully at all relevant national and international interlaboratory control studies for PBDEs in various samples like e.g. Norwegian Institute of Public Health, Quasimeme and others [6].

Results and discussion

In Table 2 the content of dry matter in soil and the content of lipids for deer liver are given. The dry matter varies widely between 26.3 and 69.2 % in the humus samples and 33,2 and 93,6 % for A-horizon soil respectively. Lipid content found in the deer liver samples shows a relative lower range between 3,5 and 5,4 %.

In Figure 1 the total for all 13 PBDE congeners in soil samples are shown for the samples originating from the 9 sites. Highest concentrations were found for all sites in the humus samples, A-horizon samples show a 5 to 10 fold lower concentration. Lowest concentrations were found in samples from Berchtesgaden and Bayerischer Wald while highest values could be detected in samples from Solling and Tertiärhügelland.

Origin	Soil Samples				Deer Liver Samples	
		Dry matter (%)		Dry matter (%)		Lipid content (%)
Solling	2002 Humus 2002 A-H	44,7 73,3	2006 Humus 2006 A-H	37,3 67,5	2001 2003 2007	4,5 5,1 5,4
Bornhöved	- 2002 A-H	- 91,3	- 2006 A-H	- 87,9	2003 2007	4,9 4,8
Pfälzer Wald	2002 Humus 2002 A-H	47,7 83,4	2006 Humus 2006A-H	52,4 81,1	2003 2007	5,0 4,9
Bayerischer Wald	2002 Humus 2002 A-H	34,8 60,5	2006 Humus 2006 A-H	28,6 53,7	2003 2007	4,7 4,8
Harz	2002 Humus 2002 A-H	26,3 51,2	2006 Humus 2006 A-H	57,3 74,0	2003 2007	5,4 4,7
Berchtesgaden	2002 Humus 2002 A-H	27,8 33,2	2006 Humus 2006 A-H	32,8 35,9	2002 2006	3,5 4,8
Tertiärhügelland	2002 Humus 2002 A-H	32,4 75,0	2006 Humus 2006 A-H	56,5 84,1	2002 2006	4,4 4,4
Warndt	2002 Humus 2002 A-H	55,5 86,1	2006 Humus 2006 A-H	61,4 79,8	2001 2005 2007	4,5 3,9 4,6
Dübener Heide	2002 Humus 2002 A-H	69,2 91,3	2006 Humus 2002 A-H	60,8 93,6	2001 2003 2007	4,0 4,4 4,7

Table 2: Dry matter and lipid content in soil samples and deer liver samples

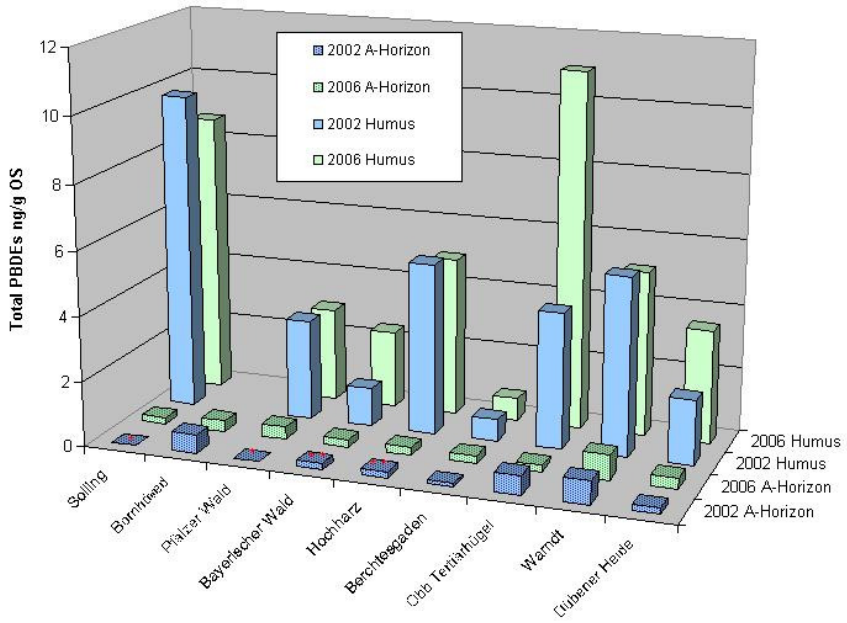

Figure 1: Total PBDEs in soil samples, ng/g original sample (OS)
*** not quantified ** excl. BDE 209**

As can be observed from the 2002 and 2006 samples, a clear time trend could not be observed. The relatively high concentration for the 2006-humus sample originating from Tertiärhügelland may be an outlier - as it is not reflected in the A-horizon samples from the same site.

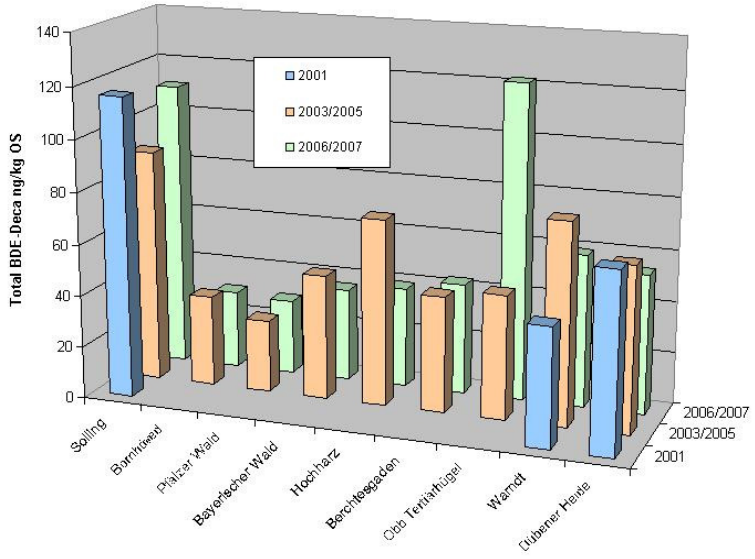

Figure 2: PBDEs in deer liver (total BDE excl. BDE#209) ng/kg original sample (OS)

In Figure 2 the total PBDE excl. BDE#209 concentrations are presented for all 9 individual sites and for the three time periods. Similar as observed for soil samples highest PBDE concentrations for deer liver are found in the Solling samples followed by Tertiärhügelland and Warndt. In general, the values range between 30 and 120 ng/kg original sample.
A tendency for declining PBDE values from 2001 to 2006/2007 can be seen for most samples from the different sites. This declining trend can not be seen for the samples from Tertiärhügelland (similar observation found for soil samples from this site).

Acknowledgements:
We acknowledge the funding of this project within the framework of the German Environmental Specimen Bank which is financed and organized by the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety and the Federal Environmental Agency.

References:

1. Schröter-Kermani C, Helm D, Herrmann T, Päpke O,(2000); The German Environmental Specimen Bank – Application in Trend Monitoring of Polybrominated Diphenyl Ethers in Human Blood, Organohalogen C. 47, 49-52
2. Rüdel H, Fliedner A, Jürling H, Müller J, Pfleger D, Stegemann D, Schröter-Kermani C, (2011); Comparison of Levels and Trends of Industrial Chemicals (PCBs, PFCs, PBDEs) in Archived Herring Gull Eggs from German Coastal Regions. *In preparation*
3. Paepke O, Schröter-Kermani C, Stegemann D, Neugebauer F, Ebsen P, (2011); Analytical Experiences with the German Environmental Specimen Bank: PBDE Time Course in Herring Gull Eggs; BFR2011, Boston, June 6./7.
4. Schröter-Kermani C, Rappolder M, Neugebauer F, (2011) PCDD, PCDF and dl-PCB in terrestrial ecosystems: Are there correlations of levels or patterns in soil and roe deer liver? *In preparation*
5.www.umweltprobenbank.de
6. Pfleger D, Schröter Kermani C, Ebsen P, Opel M, Paepke O, (2010) QA/QC-Measures for continuous monitoring of POPs in Biota samples collected in the frame of the German Environmental Specimen Bank. Organohalogen C. 72,771-774