PBDE AND PBB IN VARIOUS FISH, FISH OIL AND ENVIRONMENTAL SAMPLES OF 2010 AND 2011

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Introduction

Polybrominated diphenylethers (PBDEs) and polybrominated biphenyls (PBBs) have been widely used as flame retardants. Technical PBDE preparations are produced as mixtures of mainly penta-, octa- or decabrombiphenyl ethers (1). PBDEs and PBBs are structurally similar to other persistent environmental pollutants (POPs) like dioxins and polychlorinated biphenyls (PCBs). They are lipophilic, persistent and widespread in the environment. For a number of congeners bioaccumulation has been observed (2). Hexabromobiphenyl has been used as a flame retardant mainly in the 1970s under the technical name FireMaster FF-1 and FireMaster BP-6. Hexabromobiphenyl, Hexabromodiphenyl ether and heptabromodiphenyl ether are regulated as new POPs in the Stockholm Convention as communicated to all Parties on 26 August 2009 by the amendments to Annex A, B and C of the convention(3). This paper gives an impression of the current contamination of fish, fish oil, wastewater, soil, sediments and sewage sludge from different countries of origin.

Materials and methods

The samples reflect routine analyses performed at the Eurofins GfA Lab Service GmbH POPs competence centre in Hamburg/Germany from January 2010 to end September 2011. The sample types analysed comprise different environmental compartiments and fish and fish oil from different countries of origin. Typical fish species analysed are cod, lumpfish, haddock, redfish, pollock, orange roughy, halibut, salmon, herring, tuna, atlantic catfish, mackerel, blue whiting, capelin, trout and others. The analysis took place using highly standardized procedures (see below). The samples have been analysed for up to 24 different PBDEs and 6 different PBBs. The results shown here represent selected hexa-, hepta- and the deca-BDE (IUPAC# 153, 154, 183, 209) and one tetra and one hexa-BB (IUPAC# 52 and 153). Further analytical data on other PBDE- (e.g. IUPAC# 47 and 99) and PBB-congeners will be available at the conference.

Preparation of water samples

1 L of water is filtrated and liquid/liquid extracted using 3 times 100 mL of toluene. The organic phases are combined and dried over sodium sulphate. The filter is dried at 80°C and then extracted by soxhlet-extraction for 12 h using the toluene phase from the liquid/liquid extraction. The crude extract is evaporated to 10 mL and an aliquot taken for further analysis.

Preparation of sediment samples

Sediment and soil samples are freeze dried prior to further analysis. 5 -10 g of the dried material is extracted by soxhlet extraction for 8 h using toluene. The crude extract is evaporated to 25 mL and an aliquot taken for further analysis.

Preparation of biota and fish oil samples

Biota samples as e.g. fish are freeze dried prior to further analysis. Depending on the fat content of the material, 5 -10 g of the freeze dried material is extracted by soxhlet extraction for 16 h using hexane/acetone (4:1). The crude extract is reduced to a small volume. Fish oil is used directly and dissolved in 10 mL n-hexane.

PBDE/PBB analysis

For PBDE and PBB analysis, a mixture of ^{13}C-labelled PBDE congeners (see below) is added to the sample or extraction aliquot as quantification standard and the volume is further reduced to 0.5 – 1 mL. N-hexane and conc. sulphuric acid are added (4 mL each in case of water or sediment samples, 10mL each for fish or fish oil samples), the whole mixture is shaken for 20 min (fish, fish oil 30 min) and the phases are separated. This step is

repeated one more time. The combined hexane phases are reduced to 0.5 mL in a nitrogen stream and then cleaned by an aluminium oxide column chromatography using 2.5 g of aluminium oxide (basic, activity super I) and the following solvents:

- Conditioning with 10 mL hexane
- Fractionation with 9 mL hexane/toluene and 8 mL hexane
- Elution with 13 mL dichloromethane.

A recovery standard is added to the dichloromethane eluate and the solution is then reduced to a final volume of 50 µL.

The measurements were performed using high-resolution gas chromatography/low resolution mass spectrometry (HRGC/LRMS) using a DB-5 column for gas chromatographic separation. Quantification was performed by an isotope dilution method using ^{13}C-labeled quantification standards added before extraction.
The following internal standards were used for quantification of the PBDEs and PBBs mentioned above: $^{13}C_{12}$-BDE-47, $^{13}C_{12}$- BDE-153, $^{13}C_{12}$- BDE-183, $^{13}C_{12}$- BDE-209.
For QA/QC, recovery rates have been monitored with the recovery standard $^{13}C_{12}$- BDE-138 added before GC/MS injection. Recovery rates were accepted between 50-130%. Further QA/QC measures have been taken, e.g. batch blank preparation over the whole procedure as well as reference samples (e.g. fish reference material CIL EDF-2525). Blank values have been below the quantification limits given in table 1 and solvents and reagents were tested before use in the laboratory processes. Furthermore, the laboratory participated successfully and regularly in national and international laboratory comparison studies, e.g. Norwegian Institute of Public Health, Quasimeme and others (4). Typical limits of quantification (LOQ) for the individual PBDE and PBB congeners are shown in table 1.

Compound	LOQ in Biota [µg/kg]	LOQ water [ng/L]	LOQ in sediment [µg/kg]
BDE-154	0.03	0.3	0.05
BDE-153	0.03	0.3	0.05
BDE-183	0.05	0.5	0.08
BDE-209	1.0	5.0	3.0
BB-52	0.01	0.05	0.02
BB-153	0.03	0.2	0.05

Table 1: typical limits of quantification (LOQ) for the individual PBDE and PBB congeners

Results and discussion

A total of 2048 individual results on PBDEs #153, 154, and 183 in the matrices wastewater, soil, sediment, sewage sludge, fish, and fish oil were reported between January 2010 and September 2011. The descriptive statistics for PBDEs # 153, 154, and 183 are shown in table 2. In sum of all 3 matrices 471 results (23%) revealed results > LOQ with an innerquartile range (25% to 75%) for wastewater of 1.1 to 9.3 ng/L, for soil, sediments and sewage sludge of 0.17 to 1.3 µg/kg, and for fish and fish oil of 0.02 to 0.14 µg/kg.

	n total	n>LOQ	Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Std.Dev.
Wastewater in ng/L	665	114	70	2.3	0.35	4440	1.1	9.3	455
Soil/sediment/sewage sludge in µg/kg dw	780	189	1.3	0.41	0.03	33	0.17	1.3	3.0
Fish and fishoil in µg/kg	603	168	0.18	0.05	0.002	4.9	0.02	0.14	0.45

Table 2: statistics for PBDE concentrations of congeners #153, 154, and 183 in different matrices

A box plot for PBDE concentrations of congeners #153, 154, and 183 in fish and fish oil is presented in Figure 1. A result is defined as an outlier or an extreme value if the value exceeds 1.5 times the innerquartile range (25 – 75%) and 3 times the innerquartile range (25 – 75%), respectively.

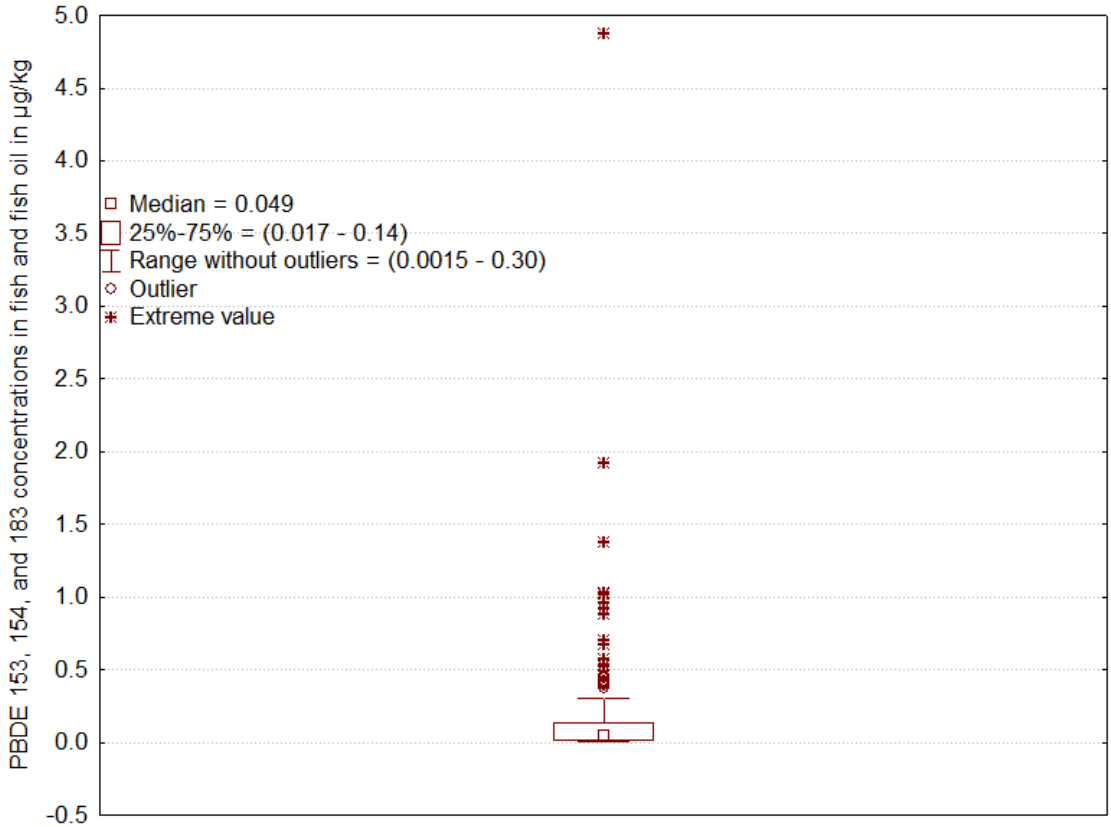

Figure 1: box plot for PBDE concentrations of congeners #153, 154, and 183 in fish and fish oil

A total of 697 individual results on BDE #209 in the matrices wastewater, soil, sediment, sewage sludge, fish, and fish oil were reported between January 2010 and September 2011. The descriptive statistics for BDE # 209 is shown in table 3. In sum of all 3 matrices 295 results (42%) revealed results > LOQ with an innerquartile range (25% to 75%) for wastewater of 17 to 168 ng/L, for soil, sediments and sewage sludge of 4.0 to 596 µg/kg, and for fish and fish oil of 0.35 to 2.9 µg/kg.

	n total	n>LOQ	Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Std.Dev.
Wastewater in ng/L	219	86	1129	72	6.0	76000	17	168	8186
Soil/sediment/sewage sludge in µg/kg dw	260	183	1260	27	1.1	70600	4.0	596	7004
Fish and fishoil in µg/kg	218	26	3.7	1.5	0.04	43	0.35	2.9	8.5

Table 3: statistics for PBDE concentrations of congener #209 in different matrices

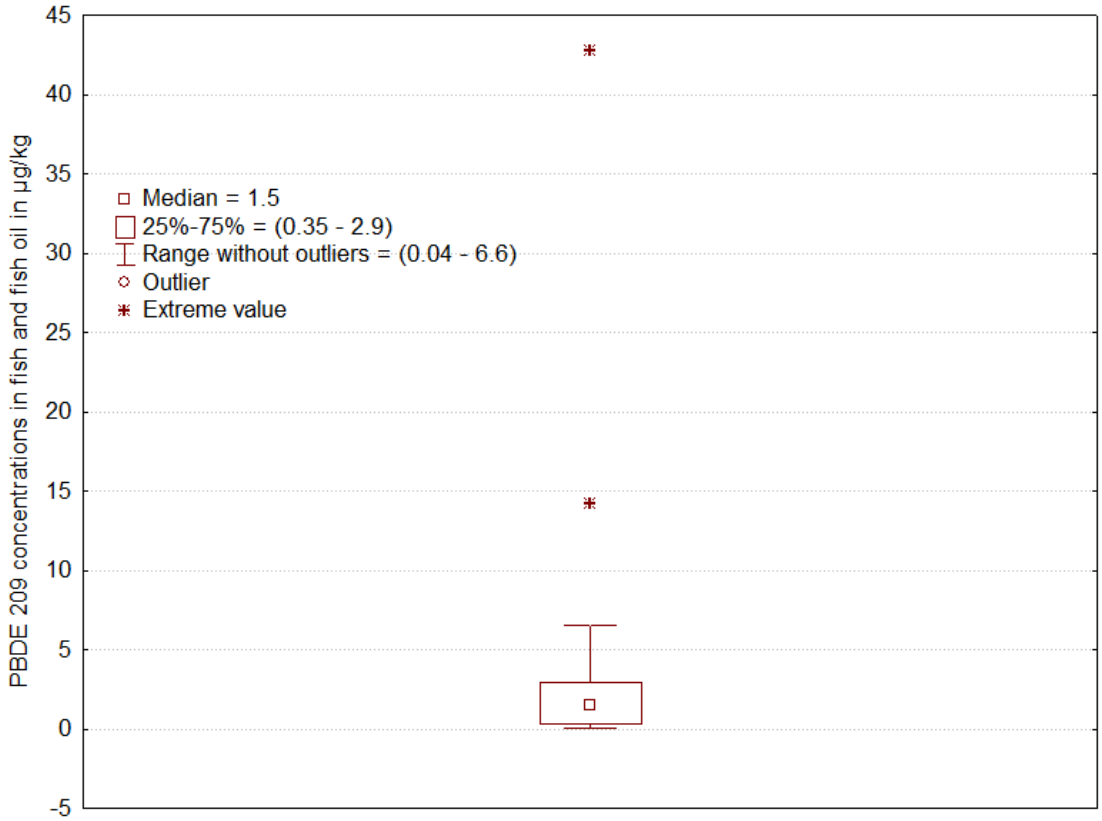

Figure 2: box plot for PBDE concentrations of congener #209 in fish and fish oil

A total of 251 individual results on BB #52 in the matrices wastewater, soil, sediment, sewage sludge, fish, and fish oil were reported between January 2010 and September 2011.
Only 1 of 151 results for wastewater was above the limit of quantification of typically 0.05 ng/L. None of the results for the other matrices could be reported above the limit of quantification.

A total of 242 individual results on BB #153 in the matrices wastewater, soil, sediment, sewage sludge, fish, and fish oil were reported between January 2010 and September 2011.
Only 2 of 142 results for wastewater were above the limit of quantification of typically 0.2 ng/L. None of the results for the other matrices could be reported above the limit of quantification.

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References:
1. Bergman A., (2000) *Organohalogen Compounds* 47: 36-40
2. de Wit C.A., (2002) *Chemosphere*. 46: 583-624
3. Stockholm Convention on Persistent Organic Pollutants, Reference: C.N.524.2009.TREATIES-4 (Depositary Notification) – http://chm.pops.int
4. Pfleger D., Schröter Kermani C., Ebsen P., Opel M., Paepke O., (2010) *Organohalogen Compounds* 72 : 771-774