OCCURRENCE OF POLYBROMINATED DIPHENYL ETHERS IN FRESHWATER FISH FROM GERMANY

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Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as additive flame retardants in polymer materials and textiles. They are persistent and lipophilic compounds and have become ubiquitous environmental contaminants found in freshwater as well as marine organisms and sediments from Europe, North America and Japan[1]. At the present time, the most frequently used PBDE is decabromodiphenyl ether (DeBDE) with a global market demand for 1999 of 54,800 tons[2]. There are only a few studies on DeBDE concentrations in the aquatic environment. It was identified in high concentrations in sediment samples from diverse locations[1] and at low levels in mussels and marine fish species from Japan[3,4]. Pike samples from Sweden contained traces of DeBDE, however, concentrations were just above the limit of detection of 100 ng/g lipid weight (lw)[5]. No current information on PBDE contamination levels of freshwater fish species from German rivers is available, neither for widespread tetra- to hexabromodiphenyl ethers nor for higher brominated congeners including DeBDE. An earlier work reported PBDE concentrations of 18-983 ng/g (lw) on a commercial formulation basis in freshwater fish from waters of North Rhine-Westphalia[6].

In this paper we report the concentrations of 11 BDE congeners in 22 bream and 5 eel samples from the river Elbe in Germany.

Materials and Methods

Bream *(Abramis abrama L.)* and eel (*Anguilla anguilla L.)* samples were collected during a fish species survey from the river Elbe upstream the city of Dresden in 2001. Muscle tissue was isolated and frozen until analysis for PBDEs. A modified extraction and clean-up procedure described previously was employed[7]. In brief, glassware was rinsed with n-hexane followed by acetone before use. Amber or aluminium foil wrapped glassware was used to prevent reductive debromination of the DeBDE exposed to sunlight. 10 to 50 g of fish tissue representing 0.3 to 1.0 g of lipid were dried with sodium sulphate and soxhlet extracted for 16 hours with toluene. The obtained lipid fraction was cleaned-up by multi-layer column chromatography (silica gel, alkaline- and acid-impregnated silica gel) and gel permeation chromatography. The final extract volume was 100 µl. Quantification of BDEs 28, 47, 66, 85, 99, 100, 138, 153, 154, 183 and 209 was performed by capillary gas chromatography-electron capture negative ionisation mass spectro-metry (GC-ECNI-MS) in the selected ion monitoring mode under the following conditions:

GC 6890+ / MSD 5973 (Hewlett Packard, U.S.A.) equipped with autosampler MPS2 (CTC Analytics AG, Switzerland) and PTV injector KAS 4 plus (Gerstel, Germany); capillary column: Rtx-CLPesticides (Restek, U.S.A.), 30 m x 0,25 mm, film thickness: 0,25 µm; pressure-pulse injection, injection volume: 2µl; carrier gas: helium; CI ion source, reagent gas: methane; ion source temperature:

210°C. PBDEs elute under these conditions between 11 and 35 minutes. For congeners with three to seven bromine atoms the most prominent ions due to bromine at m/z = 79 and m/z = 81 were recorded, while for the decabromodiphenyl ether (BDE-209) the highly specific ions at m/z = 484,7 and m/z = 486,7 were monitored. BDE-77, BDE-181 never found in environmental samples, ^{13}C-labelled BDE-209 and dibromooctafluorobiphenyl (m/z = 375 and m/z = 457) were used as internal standards and syringe standard, respectively. All standards were purchased from Promochem (Wesel, Germany) and Greyhound chromatography (Birkenhead, U.K.).

For confirmation identity of BDE-209 high-resolution mass spectrometric (HRMS) analysis was performed on three selected fish extracts in the electron impact mode using a GC-HRMS System consisting of a gas chromatograph HP 5910 coupled with a high-resolution mass spectrometer VG AutoSpec (VG, U.K) operated at a resolution of 10,000. Signals of the $[M\text{-}Br_2]^+$ cluster at masses 797.3355, 799.3334 and 801.3314 were recorded.

Results and Discussion

Concentrations of 11 BDE-congeners and total concentrations (sum of 11 congeners) in samples of bream *(Abramis abrama L.)* and eel (*Anguilla anguilla L.)* are summarised in Table 1 and 2 with information on minimum, maximum and median concentration. Figure 1 shows an example of a chromatogram of bream extract containing 18 ng/g (lw) of BDE-209.Eel and bream samples showed similar profiles of BDEs dominated by BDE-47 with higher concentrations in bream. The predominance of BDE-47 is consistent with other studies on freshwater fish species[1]. Bream collected from several sites in the Netherlands had BDE-47 concentrations of 0.2-130 ng/g dry weight (dw) with a median of 16 ng/g dw[8]. This indicates a quite similar contamination level for breams from Dutch freshwater ecosystems and those from the river Elbe. BDE-47 concentrations in bream from the river Elbe were 0.9-62 ng/g dw with a median of 8.4 ng/g dw. Sum of BDE in eel (n=5) and bream (n=22) ranged from 4 to 21 and from 26 to 728 ng/g (lw), respectively. BDE levels in eel from the river Elbe were quite low compared to those from Dutch rivers and lakes[9] Not all congeners could be detected in each of the samples but BDE-47, BDE-100 and BDE-154 were found in all bream and eel samples analysed. Other congeners were largely found at low levels or were below the limits of quantification (LOQ). 11 out of 22 bream samples contained BDE-209 in concentrations up to 37 ng/g lw. BDE-209 results of three samples (two with high concentrations and one below the limit of quantification) were confirmed by GC-HRMS analysis. In eel samples, no BDE-209 was detected. To our knowledge, this is the first report on the occurrence of decabromodiphenyl ether in freshwater fish in Europe. It has been generally thought that BDE-209 is unlikely to bioaccumulate due to its high molecular size and its extreme lipophilicity, which may impede its release from sediment particles[5]. But recent reports on higher brominated congeners including BDE-209 in marine fish species from Japan[4], in plaice (*Pleuronectes platessa*), flounder (*Platychthys flesus*) and dab (*Limanda limanda*) collected from the estuaries of British rivers[10] and these data suggest that congeners representative for technical OcBDE and DeBDE are bioavailable. Thus, biomagnification in the aquatic food chain seems to be possible. These results are consistent with a recent dietary exposure study on rainbow trout in which accumulation of BDE-209 in muscle and liver tissue has been observed[11]. It is difficult to assess the significance of BDE-209 concentrations reported as mechanisms of uptake; metabolism and toxicity are poorly understood at present. Further studies using more sophisticated and sensitive analytical methodologies are needed to get an idea on the actual occurrence of higher brominated BDEs, which continue to be widely used, in the aquatic environment.

Table 1. Concentrations of PBDEs in bream samples in ng/g (lw), n = 22, lipid concentrations varied between 0.5 and 6.6%, LOQ = limit of quantification

BDE congener	**Minimum**	**Maximum**	**Median**
BDE 28	<LOQ	7.2	0.73
BDE 47	16.4	482	127
BDE 66	<LOQ	<LOQ	<LOQ
BDE 100	3.3	123	31.8
BDE 99	<LOQ	3.1	0.49
BDE 85	<LOQ	4.1	3.0
BDE 154	2.3	95.3	18.2
BDE 153	<LOQ	17.7	4.8
BDE 138	<LOQ	4.8	4.6
BDE 183	<LOQ	0.70	0.18
BDE 209	<LOQ	37.3	0.97
Sum of PBDE	26	728	198

Table 2. Concentrations of PBDEs in eel samples in ng/g (lw), n = 5, lipid concentrations varied between 9 and 39%, LOQ = limit of quantification

BDE congener	**Minimum**	**Maximum**	**Median**
BDE 28	0.003	0.08	0.02
BDE 47	2.0	14.8	4.5
BDE 66	<LOQ	0.04	0.05
BDE 100	0.57	3.8	0.98
BDE 99	0.11	0.29	0.14
BDE 85	<LOQ	<LOQ	<LOQ
BDE 154	0.17	1.39	0.48
BDE 153	0.13	0.54	0.21
BDE 138	0.21	0.56	0.26
BDE 183	0.01	0.07	0.04
BDE 209	<LOQ	<LOQ	<LOQ
Sum of PBDE	3.6	21.4	6.3

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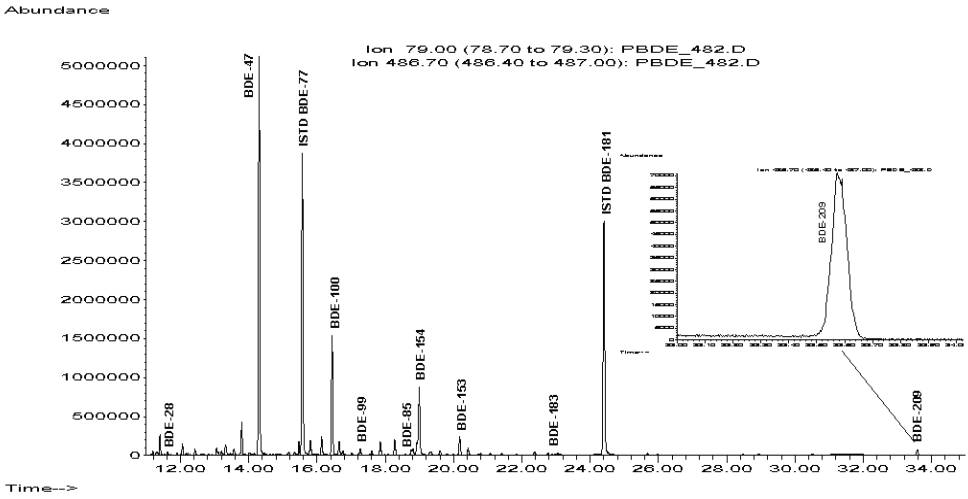

Figure1. GC-ECNI-MS chromatogram of a tissue extract from bream caught upstream the city of Dresden in the river Elbe, Germany, in April 2001. The signal at 33.6 min (m/z = 486.7) corresponds to a BDE-209 concentration of 18 ng/g (lw)

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