BROMINATED FLAME RETARDANTS IN BREAM (*Abramis brama* L.) FROM SIX RIVERS AND A LAKE IN GERMANY

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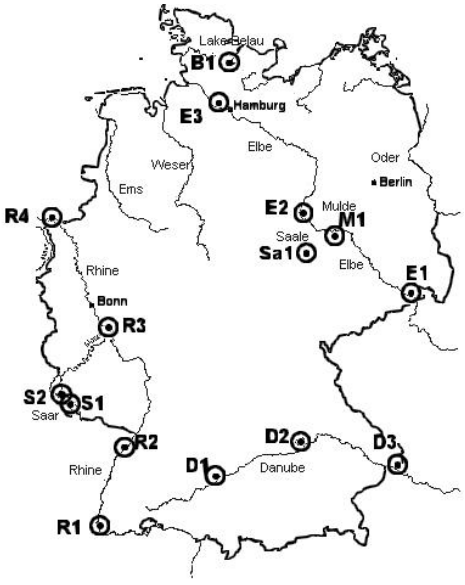
Introduction

Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and other brominated compounds are used as flame retardants in polymers, textiles, electronic boards and various other materials. Kow values and bioaccumulation factors (BAF) of brominated flame retardants (BFRs) indicate that they have the capacity to accumulate in fatty tissue and to biomagnify along the food chain. This, in combination with their persistence and adverse health effects makes them to chemicals of a high concern. To identify spatial and temporal trends and to study the fate of these chemicals in the environment and their impact on human beings, extensive monitoring in various environmental compartments as well as in human samples is required. The German Environmental Specimen Bank (ESB, www.umweltprobenbank.de) comprises archives of plant, animal and human specimens collected at various locations representing different ecosystems and levels of contamination periods, which are ideal sample materials to conduct such studies.

Here we report the results for 23 PBDE congeners, HBCD (α-, β- and γ-diastereoisomers), and six other BFRs determined in bream, one of the most common freshwater fish species in Central Europe, collected and archived by ESB from 1995 to 2009 at 14 locations along six German rivers (Rivers Saar, Rhine, Elbe, Saale, Mulde and Danube) and at Lake Belau, which served as a reference location. We used fully optimised and validated GC-ECNI-MS and LC-MS/MS methods for analyses of PBDEs/other BFRs and HBCD, respectively.

Materials and methods

Sampling has been described in detail elsewhere[1]. In brief, at least 20 breams were collected at each sampling site between middle of August and middle of October, i.e. after the spawning period.



Lake Belau	B1 -	Lake Belau
River Saar	S1 -	Güdingen
	S2 -	Rehlingen
River Rhine	R1 -	Weil
	R2 -	Iffezheim
	R3 -	Koblenz
	R4 -	Bimmen
River Elbe	E1 -	Prossen
	E2 -	Barby
	E3 -	Blankenese
River Saale	Sa1 -	Wettin
River Mulde	M1 -	Dessau
River Danube	D1 -	Ulm
	D2 -	Kelheim
	D3 -	Jochenstein

Fig. 1 Map of Germany with sampling locations

To reduce the effect of age on the BFR concentrations, we aimed at sampling specimen of the age class 8 to 12 years at all locations. Immediately after sampling and biometrical characterization, fish were dissected to obtain the muscle tissue for analysis. All further steps were performed maintaining the cold chain. Tissue samples were combined and homogenized in order to obtain annual sample homogenates. Finally, sub-samples of approximately 10 g each were stored above liquid nitrogen.
A total of 91 bream samples collected in the period 1995 to 2009 from six different German rivers and from the reference site Lake Belau (Fig. 1), were investigated.

An analytical procedure described previously has been applied with some modifications[2]. In brief, freeze-dried and homogenized muscle tissue samples representing 0.1 to 0.5 g of lipid (corresponding 0.5 to 2 g dry weight) were spiked with an internal standard mixture and extracted with toluene at 125 °C and 14 MPa (4 cycles) using an ASE 200 accelerated solvent extractor (Dionex GmbH, Idstein, Germany) followed by gel permeation chromatography and multi-layer silica gel column chromatography clean-up. The purified extracts were evaporated to a final volume of 100 µl using an automatic evaporation device (Flowtherm Optocontrol, Barkey GmbH & Co. KG, Leopoldshoehe, Germany). Quantification of 23 BDE congeners (BDE17, BDE28, BDE47, BDE49, BDE66, BDE71, BDE77, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE179, BDE183, BDE188, BDE196, BDE197, BDE202, BDE203, BDE206, BDE207 and BDE209) and six other BFRs (PBT, PBEB, HBB, BB153, BTBPE and BB209) was performed by GC-ECNI-MS in the selected ion monitoring mode under the following conditions: GC 6890+ / MSD 5973 (AGILENT, Palo Alto, CA, U.S.A.) equipped with autosampler MPS2 (CTC Analytics AG, Zwingen, Switzerland) and PTV injector CIS 4 plus (GERSTEL, Muelheim / Ruhr, Germany); capillary columns: Rtx-CLPesticide (Restek, Bellefonte, PA, U.S.A.), 30 m x 250 µm ID, film thickness: 0.25 µm (BDE209 was additionally analyzed on a 5 m x 100 µm ID capillary column, Rtx-CLPesticide, film thickness: 0.10 µm); pressure-pulse injection: 50 psi (0.8 min); injection volume: 2 µl; carrier gas: helium; CI ion source; ion source temperature: 200 °C. While for quantification of BDE 209 the highly specific ions at m/z = 484.7 and m/z = 486.7 were monitored, for most other BFRs ions specific to bromine at m/z = 79 and m/z = 81 were recorded.

For LC-MS/MS analysis of HBCD diastereoisomers, sample extracts were redissolved in methanol. LC separation was carried out using a LC-20A Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a reversed phase column Agilent ZORBAX Eclipse XDB-C18 (2.1 x 150 mm, 3.5 µm). The LC was coupled to a triple-quadrupole mass spectrometer API 4000 QTRAP (AB Sciex, Foster City, California, U.S.A.) using electrospray ionization (ESI negative) in MRM mode with two specific transitions. The $(M\text{-}H)^- \rightarrow$ Br- transition at m/z 640.8 →78.8 was monitored for the quantification of the three unlabeled HBCD isomers. The $(M\text{-}H)^- \rightarrow$ Br- transition at 652.8 → 78.8 was monitored for $^{13}C_{12}$-labelled HBCD diastereoisomers. The initial mobile phase composition of 95 % H_2O / 5 % CH_3OH was held for 0.01 min followed by a gradient to 10 % H_2O / 90 % CH_3OH within 5 min, which was held for 15 min. The flow rate was adjusted to 0.2 mL/min and the injection volume was set to 10 µL. All HBCD diasteroisomers eluted between 15 to 20 minutes.

The fluorinated brominated diphenyl ethers F-BDE28, FF-BDE47, F-BDE100, F-BDE183, FF-BDE201, F-BDE208, $^{13}C_{12}$-labelled BDE209 and the $^{13}C_{12}$-labelled α-, β-, and γ-diastereoisomers of HBCD were used as internal standards. Quality controls included recovery tests, regular analyses of procedural blanks, blind replicate samples, as well as participation in international interlaboratory studies on the determination of various PBDEs and HBCD (e.g., Quasimeme Round 60 and 62, POPs in Food 2010). Standards were purchased from Chiron AS (Trondheim, Norway) and Wellington Laboratories Inc. (Guelph, Ontario, Canada).
Extractable lipids were determined gravimetrically using the toluene extracts obtained by ASE and varied between 0.79 % and 8.4 % (median 4.2 %).

Results and discussion:

Table 1: Concentration [range (median) ng/g lipid weight] of selected BFRs in 91 bream muscle tissue samples from six German rivers and a lake collected in a sampling period from 1995 to 2009

Sampling location	BDE 28	BDE 47	BDE 49	BDE 99	BDE 100	BDE 153	BDE 154	BDE 209	∑HBCD
Lake Belau (B1)	0.32-0.71 (0.52)	8.2-17.1 (12.6)	0.79-1.24 (1.01)	<0.4-1.07 (1.07)	2.22-3.21 (2.71)	0.50-0.98 (0.74)	1.69-2.28 (1.99)	6.90-8.20 (7.55)	9.08-13.1 (11.1)
River Saar - Güdingen (S1)	10.4-56.3 (15.4)	404-1146 (427)	3.75-12.5 (6.11)	3.28-8.93 (4.61)	224-801 (353)	48.1-155 (97.3)	59.1-176 (126)	2.36-29.0 (2.95)	121-285 (198)
River Saar - Rehlingen (S2)	26.9-126 (78.2)	505-2662 (1877)	8.41-27.5 (23.3)	6.06-102 (13.0)	332-1219 (976)	95.4-238 (172)	86.3-281 (203)	2.14-5.69 (3.79)	168-272 (191)
River Rhine - Weil (R1)	1.05-5.12 (1.67)	47.7-120 (85.6)	0.56-5.10 (0.74)	0.80-1.75 (1.00)	12.1-91.1 (24.8)	3.62-8.75 (6.14)	4.35-14.1 (9.55)	1.30-9.77 (1.74)	26.6-882 (66.7)
River Rhine - Iffezheim (R2)	1.05-3.71 (2.75)	59.4-153 (98.1)	0.87-6.92 (2.50)	0.94-1.71 (1.25)	11.4-55.2 (37.6)	3.16-11.5 (8.62)	3.89-16.0 (12.1)	1.15-8.59 (2.20)	119-294 (161)
River Rhine - Koblenz (R3)	2.00-5.82 (3.48)	88.1-281 (150)	1.18-6.77 (4.82)	0.98-1.76 (1.25)	18.9-108 (43.0)	5.88-16.5 (11.1)	6.90-23.8 (11.4)	1.35-663 (95.6)	113-317 (145)
River Rhine - Bimmen (R4)	3.45-11-5 (7.33)	136-298 (235)	1.42-10.2 (8.87)	1.19-6.45 (1.74)	59.3-345 (118)	15.1-35.3 (23.1)	20.6-80.2 (27.9)	1.93-128 (5.60)	162-527 (347)
River Elbe - Prossen (E1)	3.45-11.5 (7.33)	136-298 (235)	1.42-10.2 (8.87)	1.19-6.45 (1.74)	59.3-345 (118)	15.1-35.3 (23.1)	20.6-80.2 (27.9)	1.93-128 (5.60)	162-527 (347)
River Elbe - Barby (E2)	0.79-1.77 (1.27)	32.8-81.5 (54.3)	1.24-2.15 (1.79)	0.24-1.59 (0.42)	10.8-23.9 (16.8)	3.08-4.35 (3.56)	4.39-12.3 (9.64)	1.13-19.8 (2.64)	21.4-136 (95.0)
River Elbe - Blankenese (E3)	0.49-0.93 (0.73)	14.5-30.9 (16.9)	1.11-1.70 (1.52)	0.17-2.00 (0.23)	3.21-9.27 (7.69)	0.78-1.86 (1.56)	1.39-5.24 (3.24)	0.47-30.7 (1.20)	5.74-17.2 (12.8)
River Saale - Wettin (Sa1)	1.68-6.24 (2.69)	65.7-210 (120)	1.70-4.95 (1.86)	0.50-2.12 (0.71)	16.1-70.1 (25.8)	4.80-16.9 (6.18)	7.90-41.6 (13.2)	0.79-27.9 (5.72)	50.2-1168 (361)
River Mulde - Dessau (M1)	1.20-2-06 (1.84)	49.6-130 (92.9)	1.92.3.70 (2.37)	<0.4-0.77 (0.51)	12.4-30.8 (18.9)	2.90-6.91 (5.33)	4.81-18.5 (9.61)	1.85-118 (5.17)	10.4-345 (71.7)
River Danube - Ulm (D1)	1.23-2.08 (1.62)	35.3-67.4 (59.8)	1.71-2.03 (1.73)	<0.4-0.55 (0.38)	11.1-18.8 (13.0)	2.01-4.37 (3.10)	3.22-5.95 (4.59)	0.76-0.94 (0.85)	67.2-254 (107)
River Danube - Kelheim (D2)	0.90-1.93 (1.75)	30.8-43.9 (38.9)	1.00-1.41 (1.21)	<0.4-0.57 (0.46)	8.96-16.1 (10.5)	2.30-4.28 (2.66)	3.02-6.10 (3.67)	2.21-4.95 (3.22)	46.4-130 (86.7)
River Danube - Jochenstein (D3)	0.75-2.12 (1.26)	39.9-94.2 (68.6)	0.82-1.97 (1.24)	<0.4-0.63 (0.36)	12.7-36.2 (24.4)	3.33-7.55 (5.32)	4.50-14.0 (7.19)	1.02-2.00 (1.94)	57.6-121 (94.1)

With the exception of BDE77 and BDE85 all 23 congeners analysed for were found at detectable concentrations at least in some of the 91 samples. Concentrations of BDE17, BDE71, BDE179, BDE183, BDE188, BDE196, BDE197, BDE202, BDE203, BDE206 and BDE207 were in most cases close to the limit of quantification (LoQ). The **Σ**7BDE of selected tri- to hexabrominated congeners (BDE28, BDE47, BDE49, BDE99, BDE100, BDE153 and BDE154) accounted for 14 to 99 % (median 57 %) of the total BFR content. In bream, BDE47, contributed to 11–75 % (median 58 %) to the total BDE content. A strong correlation (r^2 = 0.989) was found between the content of BDE47and the sum of all BDE congeners including BDE209. A characteristic BDE congener pattern in bream muscle tissues was observed at most sites, with BDE47 as dominant congener followed by BDE100 and BDE154. BDE99 vulnerable to metabolism in freshwater fish (Stapleton et al., 2004)[3]; (Labandeira et al., 2007)[4] was found at much lower concentrations than it would be expected from its percentage composition in the technical pentaBDE formulation. Extremely high pentaBDE concentrations were seen in bream samples from River Saar (about 10 to 100 times higher than in bream from the other screened river systems). These results were in line with pentaBDE concentrations in sediments collected at the same location (Stiehl et al., 2008)[5]. Especially bream samples from Rehlingen (S2) situated nearby an industry park showed a steady increase of pentaBDE levels since 2001. In the sample from 2009 the maximum for **Σ**7BDE of 4,400 ng/g lipid weight (lw) was found clearly indicating that this site is influenced by point sources. Similarly high pentaBDE levels ranging from 660 to 11,500 ng/g lw were recently also reported for eels collected in the River Scheldt, Belgium, one of the most polluted rivers in Europe, (Roosens et al., 2008)[6].
Hajšlová et al. (2007)[7] collected bream at 11 sampling sites located in the Elbe and Vltava (Moldau) rivers. The sum of 10 BDE congeners was in the range of 3.2 to 20.8 ng/g wet weight (median 9.1 ng/g ww), which is slightly higher than our results from River Elbe – Prossen (E1) with 0.45 to 3.0 ng/g ww (median of 2.1 ng/g ww).

The median level of BDE209 in bream over all sampling sites and the whole study period was 3 ng/g lw, which is fairly low and close to the LoQ of 1.6 ng/g lw. However, very high BDE209 concentrations exceeding 100 ng/g lw and in one case even 600 ng/g lw were found individual samples, from River Rhine (R3 and R4), River Elbe (E1) and the River Mulde (Table 1). Although the average BDE209 concentrations in breams are low some

individuals are obviously highly exposed to BDE209. This observations is in agreement with other reports on BDE209 in wildlife in which the majority of the studied individuals had low or non-detectable BDE209 concentrations, while a few were highly contaminated (Voorspoels et al., 2006)[8].

Sampling sites can be ranked according to the total BDE loads in the following order: Lake Belau << River Mulde, River Elbe, River Danube < River Saale < River Rhine < River Saar. Bream samples from the reference sites Lake Belau showed by far the lowest concentrations for all classes of BFRs (Table 1, Figure 2).

BB209 PBT, PBEB, HBB were detected in less than 10 % of the samples, while measurable BB153 and BTBPE concentrations were found 59 % and 84 % of the samples, respectively. The concentrations of the latter two compounds ranged from < 0.4 to8.9 ng/g lw and < 0.4 to 3.9 ng/g lw.

Median concentrations of the sum of HBCD diastereoisomers in bream ranged from 11 to 361 ng/g lw. Alpha-HBCD was determined in 98 % of the samples and was generally the dominant diastereoisomer. This observation is in line with previous reports on HBCD diastereoisomer patterns in fish (Allchin et al., 2003)[9]. Specimens from River Saale had the highest HBCD levels (Fig. 2) and there was a steep rise in concentration until 2007. The area is characterized by a high population density and chemical and other industries, however, no specific source of contamination could be assigned to the high HBCD levels found.

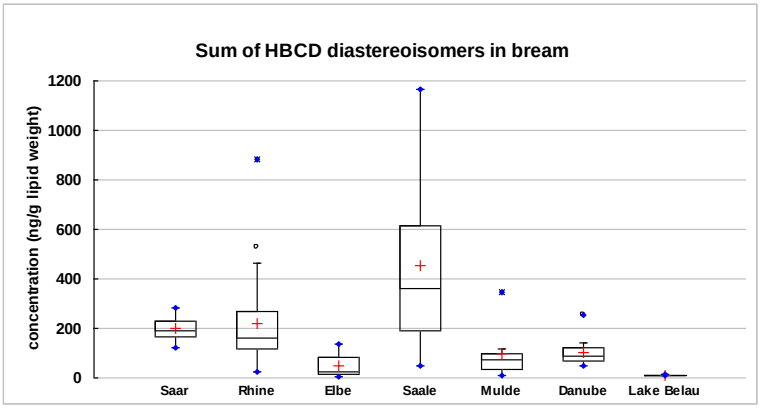


Fig. 2 Box plot of sum of HBCD diastereoisomers in bream from six German rivers and a reference lake.

+ represents the mean value,
° represents outliers, * represents extremes.

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