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Brominated Flame Retardants in Fish of Lake Geneva (Switzerland)

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Abstract Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) were determined in fish (*Salmo trutta forma lacustris*) from Lake Geneva. Brominated flame retardants were detected in all nine samples with an average concentration for the sum of BDE-28, BDE-47, BDE-49, BDE-66, BDE-99, BDE-100, BDE-119, BDE-153, BDE-154 and BDE-209 of 207 ng per g lipid weight (ng g lw^{-1}). The congener patterns were dominated by BDE-47. The average concentration of HBCD was 168 ng g lw^{-1}.

Keywords Polybrominated diphenyl ethers (PBDEs) · Hexabromocyclododecane (HBCD) · Fish · Lake Geneva

Polybrominated flame retardants (BFR) are high volume chemicals that are used to inhibit or reduce the flammability of combustible products. Polybrominated diphenylethers (PBDEs) and hexabromocyclododecane (HBCD) represent two important compound classes. PBDEs have been widely used as a flame retardant in many everyday products, such as furniture, cars, textiles and electronic equipment (De Wit 2002). Decabrominated diphenylether (DecaBDE), the main representative is added to plastics used in electrical and electronic equipment (housings of computers, TV sets etc.), the transportation sector (i.e., automotive interiors) and for construction and building (i.e., wires, cables, pipes etc.). HBCD's main use is in expanded and extruded polystyrene for thermal insulation foams for building and construction. Similar to decaBDEs, it is also applied in the backcoating of textiles, mainly for upholstery furniture. The amount incorporated in the polymers might reach up to 18% for pentabrominated diphenylethers (pentaBDEs), 15% for octabrominated diphenylethers (octaBDEs) and 16% for decaBDEs whilst percentages of HBCD in products are varying between 0.8 and 4%. For Europe, the market demand in 2001 was reported to be 9,500 t for HBCD, 7,600 t for decaBDE, 610 t for octaBDE, and 150 t for pentaBDE, respectively. These figures make HBCD and decaBDE the second most used BFRs in Europe, after tetrabromobisphenol A. The worldwide market demand for HBCD and decaBDE was estimated to further increase through 2003.

Some PBDEs exhibit physicochemical properties (environmental persistence, tendency to bioaccumulate, and potential toxicity) that would categorize them as potential persistent organic pollutants (POPs) (De Wit 2002). Penta- and octa-BDEs are subject to bans in Europe since 2004. There are indications that HBCD is being used as a replacement for these compounds. PBDEs and HBCD are both "additive" flame retardants being simply blended with the product, in contrast to "reactive" flame retardants that are covalently bound into the matrix. As a consequence, they might volatilize into the atmosphere. The result of their properties and widespread use is the ubiquitous occurrence of BFRs in the environment.

Some PBDEs have been linked to thyroid hormone disruption, neurobehavioral toxicity and, some congeners, are potential carcinogens (Darnerud et al. 2001). PBDEs are lipophilic and bioaccumulative compounds. PBDEs are removed in WWTPs by sorption onto solids (Rayne and Ikonomou 2005) and persist when discharged to the aquatic environment.

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In the aquatic environment PBDEs can accumulate in upper trophic level species like fish, some birds and humans. Potential impacts induced by these compounds reveal concerns. Therefore, the present study investigated the occurrence of PBDEs (BDE-28, 47, 49, 66, 85, 99, 100, 119, 138, 153, 154, 183 and 209) and HBCD in lake trouts (*Salmo trutta forma lacustris*) from Lake Geneva. The aims were to characterize the contamination of this species for the first time and thus to complete knowledge on concentration levels of brominated flame retardants in the aquatic environment.

Materials and Methods

Fish samples were collected in Lake Geneva. It is the largest freshwater lake in Central Europe (coordinates 46°26′N 6°33′E; surface 580 km^2; volume 89 km^3). Its main tributary is the Rhone which has its source 155 km upstream in the Alps of south-central Switzerland. The catchment area of Lake Geneva covers 7,975 km^2 with 948,240 inhabitants. It provides drinking water for 500,000 persons.

Nine male lake trouts (*Salmo trutta forma lacustris*) were captured by electric fishing in November 2004 when going up the river Aubonne to spawn. All the samples were handled carefully using gloves and immediately packed in clean deep-freezing bags. The characteristics of captured fish are given in Table 1.

The fat content of the fish was determined according to the method described by de Boer (1988). Four grams of each fish were mixed with methanol, bi-distilled water and chloroform. The results are used to express the contaminant content per gram of lipid (lipid weight).

Blank Glassware was washed with bi-distilled acetone and hexane and finally by fresh hexane before each step of the whole analysis. Solvent of the last rinse was reduced to 1 mL and injected into the GC/ECD or GC/MS to control an eventual contamination of the glassware.

All PBDEs standards and HBCD were provided by Cambridge Isotope Laboratories (CIL, Andover, USA). PBDEs standards were prepared in nonane at a concentration of 50 ± 5 mg mL^{-1} while HBCD was prepared in toluene. Working standard were obtained by diluting with isooctane (PBDEs and HBCD).

Table 1 Main characteristics of fish samples (n = 9)

	Length (cm)	Weight (g)	Age (years)	Fat content (%)
Average	57	1,793	4.2	6.4
Standard deviation	8.3	776.3	0.97	1.3

All solvents were super purity quality from Romil, Cambridge, England. Silica gel 60 (70–230 mesh) was obtained from Merck, Darmstadt, Germany. Milli-Q water was obtained from a Millipore system (Millipore, Bedford, USA).

Extraction Each fish was entirely mixed with a Büchi Mixer B-400 (Büchi, Flawil, Switzerland) in order to obtain an homogenous material.

Fifteen grams of crushed fish was added into a centrifuge tube and the sample was extracted during 10 min with the Ultra Turax placed inside of Ultrasonic bath. Solvents for extraction were: 1 × 40 mL pure acetone followed by three extractions with a mixture of acetone:hexane 25:75. Between each extraction, the sample was centrifuged at 2,500 rpm during 10 min then, top organic phase was pipetted and added into a separatory funnel contained 600 mL of MQ water and 10 mL of a saturated solution of NaCl washed with hexane. After the separation of phases, the aqueous phase was washed twice with 50 mL of pure hexane. The organic phases were combined and treated twice with 15 mL of concentrated sulphuric acid. Acid phase was washed twice with 2 × 20 mL of hexane. The combined hexanic phase was dried over Na_2SO_4 and concentrated by rotary evaporation at 40°C and 330 mbar till 1 mL.

Clean-up Silica gel was activated during 12 h at 180°C. After cooling down in a desiccator, Milli-Q water was added till a content of 3% by weight. Two glass columns were filled with silica gel. The first one for the recovery of BDE-209, the other one for the PBDEs and HBCD. Each glass column was packed dry with 3 g of deactivated silica gel, the height of column being between 14 and 15 cm. The extract was divided into two equal fractions, each fraction was transferred to the top of one column.

Hexane was added until it starts to leave the column than three separated fractions were collected: first with 16 mL of hexane, than 35 mL of hexane, and finally 50 mL of hexane:dichloromethane (v/v, 1:1). These three fractions should contain respectively, PCB, PBDEs and HBCD.

For the purification of BDE-209, two fractions were collected: first with 12 mL of hexane, (PCB) was discarded and the second one with 40 mL of hexane contains the BDE-209 (and others PBDEs).

All fractions were concentrated at 330 mbar and 40°C to 0.5 mL. Internal standard dichlorobenzyl alkyl ether (DCBE-16) was added before measurements by MDGC–ECD.

Quantification Chromatographic conditions are presented in Table 2. As some PCB and pesticides can interfere with PBDEs retention time, a multidimensional hearth-cut GCs system with ECD detectors was used for identification and quantification of BDE-28, BDE-47,

Table 2 Conditions for the chromatographic analysis

	Injection system	Column/program
PBDEs, HBCD	MDGC CP 3400 + CP3300 SPI on column 85°C (20 s) 100°C min^{-1} to 250°C (87.5 min)	First GC: DB-5 (60 m × 0.25 mm × 0.25 μm) 80°C (30 s) 30°C min^{-1} to 200°C (1 min), 10°C min^{-1} to 300°C (75 min) depending on the compound being measured
		Second GC: DB-17 (30 m × 0.25 mm × 0.25 μm) 160°C (31–42 min) 15°C min^{-1} to 280°C (21–40 min) C min^{-1} depending on the compound being measured
		ECDs temperature: 350°C
BDE-209	CP 3800, SPI on column 85°C (0.2 min) 150°C min^{-1} to 320°C (28 min)	DB-1 HT (15 m × 0.25 mm × 0.10 μm) 80°C (1 min) 30°C min^{-1} to 280°C (22.33 min)
		ECD temperature: 350°C

Table 3 Concentration of PBDEs, HBCD in fish (Lake trout, *Salmo trutta forma lacustris*) from Lake Geneva (ng g lw^{-1})

Sample	BDE-28	BDE-47	BDE-49	BDE-66	BDE-85	BDE-99	BDE-100	BDE-119	BDE-138	BDE-153	BDE-154	BDE-183	BDE-209	Sum BDE-	HBCD
Tr1	2.2	107	3.2	1	ND	57	16	0.3	ND	2.6	3	ND	2.2	195	115
Tr2	1.3	61	1.6	0.6	ND	29	7.2	0.1	ND	0.7	1.1	ND	5.1	108	51
Tr3	5.2	61	6	4	ND	29	7.2	0.3	ND	4.1	4.7	ND	9.4	131	274
Tr4	3.7	104	4.4	3.6	ND	81	20	0.2	ND	3.9	4.2	ND	11	236	207
Tr5	4.8	112	4	2.5	ND	46	14	0.3	ND	3.8	3.6	ND	10	201	112
Tr6	2.2	48	1.4	0.8	ND	24	5.2	0.4	ND	1.5	1.2	ND	5.8	90	49
Tr7	6.6	176	5.7	6.5	ND	120	27	0.4	ND	1.6	7.7	ND	24	376	324
Tr8	2.6	73	3.2	2.6	ND	41	9.6	0.4	ND	3.2	2.3	ND	5.4	143	92
Tr9	3.5	189	7.4	3.6	ND	127	31	0.5	ND	8.9	8.9	ND	8.2	387	286
Average	3.6	103	61	2.8	–	61	15	0.3	–	3.4	4	–	9	207	168
Median	3.6	104	46	2.6	–	46	14	0.3	–	3.2	3.6	–	8.2	195	115
Min	1.3	48	24	0.6	–	24	5.2	0.1	–	0.7	1.1	–	2.2	90	49
Max	6.6	189	127	6.5	–	127	31	0.5	–	8.9	8.9	–	24	387	324

LOD for BDE-85, BDE-138 and BDE-183: 0.06, 0.16 and 0.14 ng g lw^{-1}, respectively

ND not detected

BDE-49, BDE-66, BDE-85, BDE-99, BDE-100, BDE-119, BDE-138, BDE-153, BDE-154, BDE-183 and HBCD. One Varian CP-3400 (Varian AG, Zug, Switzerland) coupled to a Varian CP-3300, the first with DB-5 and the second with a DB-17 column, were used (De Alencastro et al. 2003). (DB columns obtained from Agilent technologie, Urdorf, Switzerland.) So using two columns with different polarity, peaks of the interfering compound will be better separated. BDE-209 was separated on a single column (Table 2).

Recovery Recoveries measurements were performed by spiking a fish sample with the analytes to be measured. A fish sample was bought in the market and homogenized as described for the samples. Then, three replicates were analyzed without any addition, to know the background levels of all compounds. Samples of fish were spiked with a spiking standard at four increasing concentrations: 50%, 100%, 150%, and 200% of their initial concentration. As BDE-138, BDE-183, BDE-85, BDE-119 were not present in the "non spiked sample" initial values were supposed to be the detection limit obtained with a standard solution.

Recoveries for PBDEs were in the range of 71% (BDE-138) till 98% (BDE-153). Recovery for BDE-209 was 82% and 92% for HBCD. Results presented in Table 3 were corrected for recovery rates.

Identity of PBDEs congeners was confirmed by negative ion chemical ionization mass spectrometry coupled to gas chromatography (GC-MS-NCI). Quantification was performed at *m/z* values of 79 and 81.

Results and Discussion

The PBDE congeners 28, 47, 49, 66, 99, 100, 119, 153, 154 and 209 were detected in all samples. BDE-85, 138 and 183 were below LODs. The content for the sum of PBDEs was between 90 and 387 ng g lw^{-1} (average 207 ng g lw^{-1}; Table 3). The concentration level given as the sum of BDE-

28, 47, 99, 100, 153, 154, 183 is higher by a factor of four as compared to whitefish (*Coregenus* sp.) from the lake Geneva (Zennegg et al. 2003a; Table 4). Accordingly, concentration levels in whitefish of other Swiss lakes were lower except for samples from lake Greifen which showed concentrations in the same range as the present study (Zennegg et al. 2003a). The higher burden of lake trout compared to white fish might be due to different feeding habits of the two species and its higher position in the food chain. The similar concentrations observed in whitefish from lake Greifen are probably due to the high percentage of WWTP effluents discharged into the lake. In general, the contamination level found in the present study is relatively high compared to other studies comprising data of freshwater fish (Vives et al. 2004; Table 4). Concentrations in fish from remote mountain areas found by Schlabach et al. (2004) and Stone (2006) were higher by one order of magnitude or even more. This shows that high burdens in organisms can even occur at locations where a moderate contamination level is expected.

The congener profiles were dominated by BDE-47, 99 and 100. They represented 51%, 28% and 7%, respectively, of the total amount of PBDEs (Fig. 1). This complies with data on whitefish (*Coregonus* sp.) originating from Lake Geneva (Zennegg et al. 2003a, b). As in previous studies (De Wit 2002), BDE-47 was the most abundant congener among the three prevailing compounds and the ratio BDE-47:99 $\sim$2:1. This contrasts to the profile of their main source, the penta technical products with a characteristic ratio of BDE-47:99 of $\leq$1:1 (La Guardia et al. 2006) and matrices representative for emissions thereof such as sewage sludge (Kupper et al. 2008) or WWTP effluents exhibiting similar patterns (Rayne and Ikonomou 2005). This might be explained by degradation of BDE-99. Stapleton et al. (2004) studied the debromination of BDE-99 in caged carp following dietary exposure and observed significant debromination converting BDE-99 to BDE-47. The results of the present study and analyses of brown trout (Vives et al. 2004) and white fish (Zennegg et al. 2003a) indicate that degradation might occur in other fish species as well. BDE-183 which regularly occurs in environmental samples was not detected in lake trouts. Debromination of BDE-183 to BDE-154 as suggested by Stapleton et al. (2004) might be an explanation. BDE-209 is the prevailing BDE used in Europe. Its fate in WWTPs and analyses of sewage sludge indicate that it is the dominating congener ending up in the aquatic environment (Kupper et al. 2008; Rayne and Ikonomou 2005). However, BDE-209 was of minor importance contributing 5% to the total PBDE amount in fish. This discrepancy might be explained by its low bioavailability due to a logK_{OW} of 10 and thus strong sorption onto solids.

HBCD was detected in all samples at concentrations between 49 and 324 ng g lw^{-1} (average 168 ng g lw^{-1}; Table 3) which is similar to those observed for the sum of PBDEs. This is in line with former studies (Eljarrat et al. 2004; Schlabach et al. 2004; Zennegg et al. 2003b). The average concentration is higher by a factor of six as compared to whitefish (*Coregenus* sp.) from the lake Geneva (Zennegg et al. 2003b). Concentration levels of other studies are highly variable (Table 4).

In general, it is difficult to compare the levels of contamination in fish between different studies. In the present work, the entire fish were analyzed whilst concentrations in fillet or in liver are usually reported in the literature.

Measuring pollutants in fillet (edible muscle tissues) can give useful information on contamination of food for humans while measuring pollutants in the entire fish is appropriate for ecological (food chain) studies. Stone (2006) has shown that concentrations of PBDEs in different parts of Chinook salmon differ with higher burdens in the whole body compared to the fillet with skin or without skin (Table 4). Fish of the present study were probably much bigger (average 57 cm and 1,793 g) than in other studies. Differences might occur between species due to distinct habits and feeding systems. Trouts are top predators and thus on a higher trophic level increasing biomagnification compared to species such as white fish or roach feeding on crustacean or invertebrates. Bioaccumulation is different between genre, male or female. In our work, we selected specifically 9 large male trouts among those captured by electrical fishing. Additionally, the fat content varies among species as their metabolism. This might lead to highly variable uptake and elimination rates of lipophilic compounds. Moreover, fillets have a lower lipid content than the entire fish. In the present study, the average percentage of lipids was different compared to other studies. These interrelations are not sufficiently elucidated at present time rendering appropriate interpretation of varying contamination levels and profiles of contaminants in different fish species difficult.

Previous studies showed that PBDE levels increased with the age of the barbel (*Barbus graellsi*) (Eljarrat et al. 2004). Fish length is directly related to fish age (Labandeira et al. 2007) and it is therefore expected that larger fish exhibit higher contents of persistent compounds such as PBDEs or HBCD due to longer exposure to the compounds. In the present study, no clear relationship was found between length or age of the fish and the concentration levels of any of the compounds. Results from the literature are ambiguous. In contrast to Eljarrat et al. (2004), Labandeira et al. (2007) did not find a correlation between PBDE levels and the age of fish (i.e., feral carp, *Cyprinus carpio*).

The occurrence of BFR in Geneva lake was investigated using fish (*Salmo trutta forma lacustris*). The results of this study are consistent with previous research reported that

Table 4 Concentrations of PBDE and HBCD in fish obtained within the present study compared to data from the literature

Location	Species	Matrix	Sum of PBDEs (ng g lw^{-1})	Reference
Lake Geneva (Switzerland)	Lake trout (*Salmo trutta forma lacustris)*	Entire fish	82–367[a]	This study
Lake Geneva (Switzerland)	White fish (*Coregonus* sp.)	Fillet	44[a]	Zennegg et al. (2003a)
Lake Greifen, Biel, Lucerne, Zürich, Neuchatel, Constance, Thun (Switzerland)	White fish (*Coregonus* sp.)	Fillet	36–165[a]	Zennegg et al. (2003a)
Farmed fish (Switzerland)	Rainbow trout (*Oncorhynchus mykiss*)	Fillet	12–24[a]	Zennegg et al. (2003a)
Lake Mjøsa (Norway)	Brown trout (*Salmo trutta*)	Whole homogenized fish or fish muscle	5,456[a]	Schlabach et al. (2004)
	Smelt (Osmerus eperlanus)		2,432[a]	
	Vendace (*Coregonus albula*)		1,215[a]	
Clackamas River (Northwest Oregon)	Chinook salmon (*Oncorhynchus tshawytscha*)	Whole body tissue (WB)	2300[b]	Stone (2006)
		Fillet with skin (FS)	1,800[b]	
		Fillet without skin (FNS)	1,500[b]	
Remote Lake Fergusson, Greenland	Arctic char (*Salvelinus alpinus*)	Liver	0.90[c]	Vives et al. (2004)
		Fillet	0.31[c]	
High mountain lakes, Europe	Brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*)	Liver	0.11–1.3[c]	Vives et al. (2004)
		Fillet	0.070–0.73[c]	
Lochnagar, high mountain	Brown trout (*Salmo trutta fario*)	Liver	11[c]	Vives et al. (2004)
			HBCD (ng g lw^{-1})	
Lake Geneva (Switzerland)	Lake trout (*Salmo trutta forma lacustris*)	Entire fish	49–324	This study
Lake Geneva (Switzerland)	White fish (*Coregonus* sp.)	Fillet	25	Zennegg et al. (2003b)
Lake Greifen, Zürich, Neuchatel, Zug, Sempach (Switzerland)	White fish (*Coregonus* sp.)	Fillet	48–210	Zennegg et al. (2003b)
Lake Mjøsa (Norway)	Lake trout (*Salmo trutta forma lacustris)* vendace (*Coregonus albula*),smelt (*Osmerus eperlanus*), perch (*Perca fluviatilis*), pike (*Esox lucius*)	Whole body or muscle fillets (pooled samples)	90–880	Schlabach et al. (2004)
Cinca River (Spain)	Barbel (*Barbus graellsi*)	Muscle tissue	ND–750[d]	Eljarrat et al. (2004)
River Viksan (Sweden)	Pike (*Esox lucius*)	Muscle tissue	<50–8000[d]	Sellström et al. (1998)

[a] PBDE congeners considered: 28, 47, 99, 100, 153, 154, 183

[b] PBDE congeners considered: 47, 49, 99, 100, 154

[c] PBDE congeners considered: 28, 47, 99, 100, 153, 154

[d] Given as wet weight

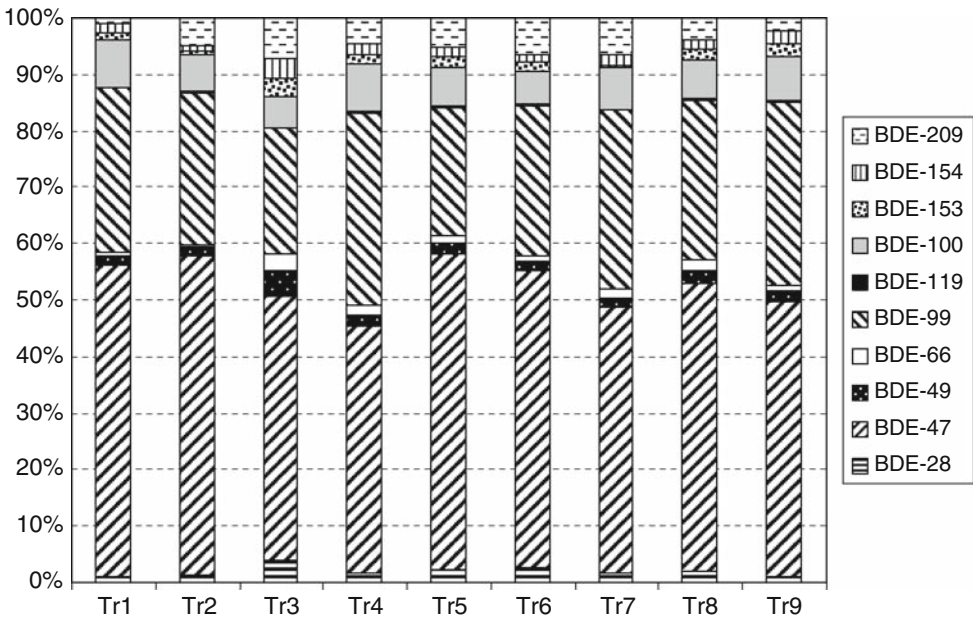

Fig. 1 Congener profiles of PBDEs in Lake trout (*Salmo trutta forma lacustris*) from Lake Geneva

BFR contamination of the aquatic environment occurs on a worldwide scale. No relationship was found between length or age of the fish and the concentration levels of any of the compounds.

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