LEVELS OF POLYBROMINATED DIPHENYL ETHERS (PBDEs) IN FISH FROM THE GREAT LAKES AND BALTIC SEA

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Introduction

Flame retardants are used as additives to plastic and textile materials to lower the risk of ignition and slow down the progression of a potential fire. A large number of organic flame retardants, e.g. halogenated substances and a variety of phosphorous containing compounds, and inorganic substances are produced for applications as flame retardants (1). Among these several brominated flame retardants (BFR) have been detected as persistent and bioaccumulating contaminants in the environment such as pentabromotoluene (2), polybrominated biphenyls (PBBs) (3), polybrominated diphenyl ethers (PBDEs) (4, 5) and hexabromocyclododecane (6).
The environmental contamination by PBDEs was first reported in pike (*Esox lucius*) from a river in the western part of Sweden (7). Since then, several reports on PBDEs in wildlife have been published (8, 9). PBDEs are also present in human blood (10) and mothers milk (11). So far the concentrations detected in wildlife and humans have been reported to be below the concentrations of PCBs and 4,4'-DDE. However, increasing concentrations of PBDEs were recently reported in mothers' milk sampled in Sweden 1972-1998 (11).
The aim of the present study was to analyze for and compare PBDEs in two species of salmonid fish, from the Great Lakes region in North America and the Baltic Sea region of Northern Europe.

Material and Methods

Chemicals: The following compounds were used as surrogate standards: 2,2',5,6'-tetraCB (CB-53), 2,2',4,5',6-pentaCB(CB-103), 2,2',3,3',4,5,5',6-octaCB (CB-198). All PCB congeners were either purchased from Promochem AB (Ulricehamn, Sweden) or synthesized according to Sundström (12). The polybrominated diphenyl ether standards used were: 2,2',4,4'-tetraBDE (BDE-47), 2,2',4,4',5-pentaBDE (BDE-99), 2,2',4,4',6-pentaBDE (BDE-100), 2,2',3,4,4',5-hexaBDE (BDE-138, surrogate standard), 2,2',4,4',5,5'-hexaBDE (BDE-153) and 2,2',4',5,6'-BDE (13, 14). 4,4'-DDT, 4,4'-DDE and hexachlorobenzene (HCB) were purchased from Aldrich.
Instruments: The high preformance liquid chromatographic (HPLC) system consisted of pump and UV-detector from Varian Chromatography System (CA, USA). A nitrophenylpropyl derivatized silica column Nucleosil-NO_2 (5 mm, 250 mm x 4.6 mm, 5cm) Jones Chromatography Ltd. (Hengoed, Mid Glamorgan, Wales) was used for HPLC separation.
The gas chromatography (GC) was performed on a Varian 3400 equipped with an electron capture detector (ECD), Varian Chromatography Systems (CA, USA) and a DB-5 capillary column (30m, 0.25 mm i.d., 0.25 μm film thickness) from J&W Scientific (Folsom, CA, USA). Temperatures of

injector and detector were 250^{o} and 360^{o}C, respectively. The GC temperature was programmed: 80^{o}C (2min), 10^{o}C min $^{-1}$ to 300^{o}C (10min). The carrier gas was hydrogen.
Gas chromatography/mass spectrometry (GC/MS) was performed on a Finnigan TSQ 700, provided with a Varian 3400 gas chromatograph equipped with a DB-5 MS column (details as described above) and helium as carrier gas. The injector and transfer line temperatures were 260^{o}C and 290^{o}C, respectively. Electron capture negative ionization (ECNI) with methane (AGA, Stockholm 4.5) as the reagent gas was used. The electron energy was 70eV and the temperature of ion source was 150^{o}C.
Samples: Six female steelhead trout (*Oncorhynchus mykiss*) were collected from lake Michigan at Kewaunee, WI in September 1995 and kept at the Wisconsin Department of Natural Resources Kettle Moraine fish hatchery prior to sampling in February 1996 when the eggs were stripped. Baltic female salmon (*Salmo salar*) from River Dalälven were sampled in late November 1995 when the eggs were stripped, for a more detailed description see Asplund *et. al.,*1999 (8). Muscle samples were collected from both species. The lipid content in steelhead from muscle was 1,4 % S.D.± 0.36 and in salmon 3,7 % S.D. ± 0,5
Clean-up and analysis: Muscle samples (about 10 g) where extracted and treated with sulfuric acid by a method similar to that described by Jensen *et al.* (15, 16). The steelhead trout samples were further fractionated by HPLC using a Nucleosil NO_2 nitrophenyl propyl silica column as described earlier (16, 8). Two fraction were collected. The first fraction included PCB, HCB and DDE. The second fraction, a back flush-fraction, contained the PBDEs. All Baltic salmon samples, were only treated with concentrated sulfuric acid and not fractionated by the HPLC.
Quantification: The PCB-fraction were analyzed GC/ECD and quantified against A50 (17). DDE, DDT and HCB were quantified by use of a pesticide standard. The PBDEs were analyzed by GC/MS using ECNI for detection of bromide ions (m/z 79, 81). The PBDEs were quantified against individual authentic PBDE standards.

Result and Discussion
The concentrations of six PBDE congeners, six PCB congeners, 4,4'-DDT, 4,4'-DDE and HCB in muscle of steelhead trout from Lake Michigan and salmon from the Baltic Sea are shown based on fresh weight (f.w.), (Table 2a) and on lipid weight (Table 2b). These tables also include data on the concentrations of sPBDEs and sPCB

Table 2. Concentrations of organic pollutants

Fish species	Steelhead trout, muscle		Baltic salmon, muscle		Steelhead trout, muscle		Baltic salmon, muscle	
	ng/g fresh weight		*ng/g fresh weight*		*ng/g lipid weight*		*ng/g lipid weight*	
Compound	**Mean** *n=6*	**S.D.**	**Mean** *n=8*	**S.D.**	**Mean** *n=6*	**S.D.**	**Mean** *n=8*	**S.D.**
CB-101	29	12	17	4,2	2000	520	310	57
CB-118	33	14	18	7,1	2300	680	330	120
CB-138	47	19	46	12	3300	920	840	170
CB-153	42	16	55	16	2900	850	1000	250
CB-170	6,8	2,6	10	3,9	480	130	180	65
CB-180	9,5	3,5	20	7,8	670	170	370	130
BDE-28	1,5	0,55	0,13	0,06	110	35	3,5	1,5
BDE-47	23	7,5	4,2	1,5	1700	760	110	34
BDE-99	7,9	2,8	1,3	0,31	600	350	35	6,1
BDE-100	4,8	1,5	0,95	0,21	360	150	26	4,0
BDE-153	1,5	0,50	0,12	0,05	110	49	3,2	1,1
BDE-154	2,7	1,1	0,22	0,05	200	120	6,0	0,9
HCB	0,71	0,3	5	1,7	48	10	95	21
DDE	130	60	170	53	9200	3400	3100	790
DDT	11	6,1	19	7,1	700	260	340	91
sPCB*	170	6,6	170	5	12000	340	3000	72
sPBDE**	41	8,2	6,9	0,55	3000	280	180	13

a. Fresh weight basis — b. Lipid weight basis

* sPCB = sum of CB-101, -118, -138, -153, -170, -180
** sBDE = sum of BDE-28, -47, -99, -100 -153, 154
Numbers according to Ballschmiter *et al*. (18)

Both fish species were collected right before spawning, and thus had a low lipid content compared to non-reproducing fish since salmonid fish do not feed prior to spawning. It is therefore reasonable to believe that the total amount of fat in the fish is lower than normal and thus the concentrations of lipophilic organic contaminants should be elevated in comparison to the normal situation. The Steelhead trout had also a lower lipid content in the muscles than the Baltic salmon which has to be considered when comparing levels between the two species.
The concentrations on a fresh weight basis of sPCB, 4,4'-DDT, 4,4'-DDE in Steelhead trout from Lake Michigan were similar or slightly lower than in the Salmon from the Baltic sea. The HCB concentrations were more than five times higher in the Salmon than in the Steelhead trout. In contrast, the levels of total PBDEs was approximately six times higher in the Steelhead trout than in Baltic salmon on fresh weight basis. Each one of the PBDE congeners analyzed was higher in the Lake Michigan fish (Table 2a). On a lipid weight basis this difference was even more pronounced

(cf. Table 2b). Among the PBDE congeners, BDE-47, BDE-99 and BDE-100, make up for approximately 90% of the total level of PBDEs. No obvious differences were observed in the relative concentrations of any the PBDE congeners as the two species were compared. It is notable that the concentration difference between even the most persistent PCB congeners, such as CB-138 and CB-153, is small compared to the major PBDE congener, BDE-47 (Table 2a and 2b). There is only a factor of two between these compounds in the Steelhead trout while there is a one order of magnitude difference in the Salmon. It is obvious that the use of PBDEs as flame retardants now have reached a point where the levels in at least certain species, are in the same range as many of the PCB congeners.

In conclusion, the results strongly indicate that PBDEs are major environmental contaminants in North America and Northern Europe. Consequently, further efforts must now be made worldwide to determine geographical and temporal trends in wildlife, at least in species at high trophic levels.

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References

1. Environmental Health Criteria 162: Brominated Diphenyl Ethers: World Health Organization, Geneva, **1994**
2. Mattsson P.E., Nordsttröm Å and Rappe C. *Journal of Chromatography* **1975**, 111,209
3. Wolff M, Henry A, Anderson A, Camper F, Nikaido M, Daum S, Haymes N, Selikoff I. Jurnal of Environmental Pathology and Toxicology **1979**, 2:1397
4. Sellström U. (*Licentiate thesis*), Polybrominated diphenyl ethers in Swedish environment, Stockholm University, **1996**, ISBN 1103-341X
5. de Boer. (*Thesis*), Analysis and biomonitoring of complex mixtures of persistent halogenated microcontaminants, Vrije Universiteit, **1995**, ISBN 90-9007970-x
6. Sellström U, Kierkegaard A, de Wit C and Jansson B; *Environ Toxicol Chem.* **1998**, 17, 1065
7. Andersson Ö, Blomkvist G. Chemosphere **1981**, 10, 1051
8. Asplund L, Athanasiadou M, Sjödin A, Berman Å and Börjeson H; *Ambio*, **1999**, 28, 67
9. Lindström G, Wingfors H, Dam M and v. Bavel B Arch Environ Contam Toxicol. **1999,** 36, 355
10. Sjödin A, Hagmar L, Klasson-Wehler, Björk J and Bergman Å. Neutral and phenolic organohalogen contaminants in human plasma related to intake of Baltic fish. Manucript.
11. Norén K and Meironyté D; Organohalogen Compounds. **1998**, 38,1
12. Sundstöm, G. *Acta. Chem. Scand* **1973**, 27,600
13. Örn U, Eriksson L, Jakobsson E and Bergman Å. *Acta Chemica Scandinavia* **1996**, 50, 802
14. Marsh G, Hu Jiwei, Jakobsson E, Rahm S and Åke Bergman. *Environ Sci Technol* In press
15. Jensen S, Reutergårdh L and Jansson B. FAO/SIDA *FAO Fish. Tech.* **1983** *Pap* 212, 21
16. Olsson A and Bergman Å. *Ambio* **1995,** 24, 119
17. Schultz D.E, Petrick G and Duincker J.C. *Environ. Sci. Technol.* **1989,** 23, 852
18. Ballschmiter K, Mennel A and Buyten J; *Fresenius J. Anal. Chem*. 1993, 346,396