Organohalogen Compounds, Volume 61, Pages 279-282 (2003)

POLYBROMINATED DIPHENYL ETHERS (PBDEs) IN MARINE FISH SPECIES OF THE BELGIAN NORTH SEA AND THE WESTERN SCHELDT ESTUARY: LEVELS, PROFILES, AND DISTRIBUTION

Stefan Voorspoels, Adrian Covaci, Paul Schepens

Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

Introduction

Brominated flame retardants (BFRs), and especially polybrominated diphenyl ethers (PBDEs), are widely used chemicals to improve fire safety in both commercial and domestic applications. The worldwide market demand for PBDEs in 2001 was estimated at 67,390 metric tons.[1,2] The increasing environmental levels resulting from this massive use, have caused these compounds to receive growing attention during the past years.[3]

These compounds are lipophilic and extremely resistant to degradation, which leads to magnification throughout the food chain[1]; especially aquatic organisms are very efficient in accumulating these compounds.[4] Animals that are higher on the food web ladder, and humans, who are on top, can accumulate substantial amounts of these compounds. Toxicological studies have demonstrated that BFRs can cause serious health effects such as thyroidogenic and estrogenic interference and dioxin-like activity.[1,5]

In this work, various fish species from the Belgian North Sea (BNS) and the Western Scheldt Estuary (SE) were analyzed for selected PBDEs. The sampling locations were chosen based on the presence of a BFR production plant at Terneuzen (located on the SE - the Netherlands), the highly industrialized nearby Antwerp harbor and the textile industry further upstream the river. Additional interest is generated by the fact that the Scheldt discharges into the North Sea, which is a very extensively exploited fishery region. Pollutants, such as PBDEs, coming from the Scheldt will be dispersed in the narrow Southern North Sea basin, where they can accumulate

Materials and Methods

Locations and species. Selection of the species was based upon their occurrence in space and time at the selected sampling locations. Seven locations were selected in the BNS and 9 locations in the SE (Figure 1). Three flatfish species (common sole (*Solea solea*), dab (*Limanda* limanda), and plaice (*Pleuronectus* platessa)) and 2 gadoid fish species (whiting (*Merlangius merlangus*) and bib (*Trisopterus luscus*)) were sampled at these locations. Sampling campaigns took place during October and November 2001, using the research vessel *Zeeleeuw*, provided by the Flanders Marine Institute (VLIZ). Preliminary sample pre-treatment steps were done on board and they included species determination, washing with clean water, dissection and storage of the excised muscle and liver samples in hexane pre-washed glass recipients at –20°C until analysis.

Targeted compounds. Based on reported abundance and toxicity, the following BDE-congeners (IUPAC numbering) were targeted for analysis: 28, 47, 99, 100, 153, 154, and 183.[3,6] Polybromobiphenyl (PBB) 103 was used as internal standard.

Sample preparation. Prior to analysis, the samples were defrosted and homogenized, using a high-speed blade-mixing device. After homogenization, two identical composite samples of each species, location and tissue were created. Pools consisted of 3 to 6 individuals. Exceptionally a

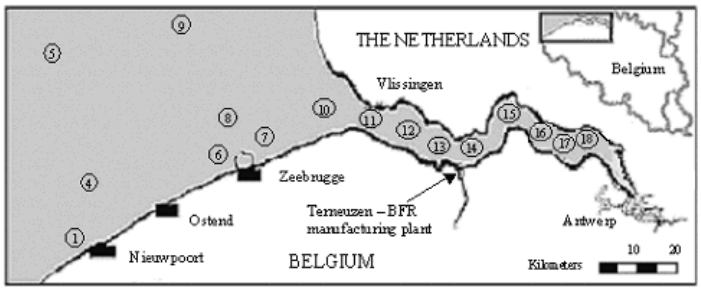

Figure 1. Sampling locations in BNS and SE.

pool consisted only of 1 or 2 individuals, for dab from location 14, and sole from locations 15 and 16, respectively.

The method used to clean up the samples has been previously described and validated and is briefly presented below.[7] Between 1 and 10g of homogenized sample was dried with approx 15g anhydrous sodium sulfate, spiked with internal standard PBB 103 (between 1 and 20 ng, depending on the pollution load of the sample) and extracted for 2,5 h by hot Soxhlet with 100 ml hexane/acetone (3/1; *v/v*). After lipid determination (performed on an aliquot of the extract), the extract was cleaned-up on 8g of acidified silica. After elution with 15 ml hexane, the cleaned extract was concentrated to approximately 80 µl.

Chemical analysis. All analyses were performed using a HP 6890 GC (Palo Alto, USA) connected via direct interface with a HP 5973 mass spectrometer. A 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Belgium) was used with helium as carrier gas at a constant flow of 1.0 ml/min. The oven temperature program was starting from 90°C, kept for 1.5 min, then with 30°C/min to 180°C, held 0.5 min, with 5°C/min to 270°C, held 0.5 min, and further with 25°C/min to 290°C and held 15 min. One µl of the cleaned extract was injected into a programmable temperature vaporizer (PTV). The injector was operated in solvent vent mode (injector temperature: 90°C, held for 0.05 min, then with 700°C/min to 280°C, and kept for 25 min; vent flow was set at 100 ml/min and the purge vent opened at 1.5 min). The mass spectrometer was operated in electron capture negative ionization (ECNI) in the selected ion-monitoring (SIM) mode at the *m/z* = 79 and 81. Methane was used as moderating gas and the ion source, quadrupole, and interface temperatures were 250, 150, and 300°C, respectively. Dwell times were set at 10 ms.

Quality control: Sample identification was based on retention times and peak shape. The quality control was done by regular analyses of procedural blanks, solvent blanks, spiked samples and blind duplicate samples.

Results and Discussion

Detected PBDE congeners and levels. In 80% of the BNS samples and in 95% of the SE samples, all congeners could be measured. Results per species and per location are presented in Table 1. PBB 153, the most abundant PBB, could not be detected in any sample.

For each species, levels of PBDEs were statistically higher for all SE locations when compared to the BNS locations. Regression coefficients between concentration found in SE samples and distance to Antwerp were found to be between R^2=0.58 and R^2=0.77 ($p<0.05$). The low mathematical correlation can be explained by the migratory character of fish and by the tidal action in the SE.[8] In general, it can be said that the geographical trend is highly similar for all species. These observations might suggest a rising trend in total PBDE concentration related to one or more point sources somewhere upstream the Scheldt, as already claimed by other studies.[9]

For the BNS area, locations 1, 4, 5, and 9 generally show the lowest concentrations. BDE levels of locations 6, 7 and 8, which are located in the vicinity of the Zeebrugge harbor, are more elevated than those of the other BNS locations. This can be related to industrial and dredging activities in

Table 1. Sum of 6 PBDE congeners (expressed in ng/g ww) for each species, tissue and location and lipid content of different tissues (expressed in mean ± se).

location		dab liver	dab muscle	plaice liver	plaice muscle	bib liver	bib Muscle	sole liver	sole muscle	whiting liver	whiting muscle
	1	6.94	0.26	5.21	0.32	71.56	0.28	4.83	0.33	24.12	0.14
	4	7.13	0.25	3.77	0.06	-	-	-	-	19.66	0.22
	5	2.81	0.15	-	-	-	-	1.17	0.08	-	-
BNS	6	-	-	-	-	108.24	0.22	8.17	0.40	89.13	0.92
	7	-	-	-	0.44	97.17	0.38	0.84	0.73	89.91	0.53
	8	5.96	0.40	37.89	0.94	27.84	0.76	2.56	0.55	24.47	0.44
	9	12.02	0.39	3.12	0.09	-	-	1.02	0.10	-	-
	10	-	-	-	-	247.63	0.86	-	-	75.23	1.04
	11	-	-	-	-	205.77	0.78	15.02	0.08	-	-
	12	-	-	-	-	173.79	0.88	-	-	-	-
	13	-	-	94.30	-	638.80	2.19	46.76	6.45	-	-
SE	14	18.61	0.66	304.34	6.13	537.46	1.90	44.45	5.09	393.04	2.37
	15	-	-	-	-	983.95	5.58	37.70	6.29	278.60	1.04
	16	-	-	74.35	-	-	-	90.10	6.87	236.44	3.00
	17	-	-	-	-	-	-	93.31	5.29	-	-
	18	-	-	-	-	-	-	-	-	-	-
lipid content		35.4 ± 2.7	1.8 ± 0.1	26.7 ± 4.0	0.8 ± 0.1	55.1 ± 2.2	0.4 ± 0.0	14.8 ± 2.4	1.0 ± 0.1	34.0 ± 4.0	0.5 ± 0.0

- = not available; BNS = Belgian North Sea; SE = Scheldt Estuary

the harbor of Zeebrugge, but is more probably due to the influence of the discharge plume of the Scheldt into the North Sea, which can reach as far as Ostend.[8] From location 10 onwards, which can be considered the beginning of the SE, PBDE levels increase very rapidly towards Antwerp; concentrations up to 984 ng/g ww were observed for bib liver tissue.

Observed profiles. BDE 47 was the most abundant analyte in all samples analyzed. BDE 47 contribution ranged between 43 and 75 %. The general order of decreasing contribution to the total load is BDE 47 > BDE 100 > BDE 99 > BDE 153, BDE 154 > BDE 28, as has been observed previously by others[10]; BDE 47, BDE 99, and BDE 100 make up about 90% on average of the total PBDE load.

For several SE samples, this general order was not followed and statistically significant higher contribution of BDE 99 could be observed. Since bioavailability of tetra- to hexa- substituted congeners can be assumed equal in marine organisms, metabolic discrepancies are most probably the basis of the profile differences observed in this study.[11]

Liver accumulation. Preferential accumulation of PBDEs in liver was estimated based on the following formula: Total concentration in liver/(Total concentration in liver + Total concentration in muscle). A ratio of 0.5 means that no preferential accumulation is observed. Higher or lower ratios indicate preferential liver or muscle accumulation, respectively. For dab, plaice, bib, and whiting, ratios of 0.57 ± 0.02 (n=7), 0.55 ± 0.04 (n=8), 0.64 ± 0.04 (n=11), and 0.67 ± 0.03 (n=10) were found when the formula was applied to the sum of PBDEs, respectively. The same ratio was found for whiting by Boon et al.[10] This can indicate that these species tend to accumulate the targeted PBDE congeners selectively in their liver. For sole however, a ratio of 0.47 ± 0.03 (n=13) was found. This low ratio may found the hypothesis that this species might not store PBDEs preferentially in its liver when compared to muscle, as do the other fish in this study.

In pike liver, active enrichment for BDE 47 was seen, which was related to the detoxifying role of the liver in xenobiotic metabolization processes and not solely to the passive redistribution governed by lipid content.[12] In our study, liver accumulation could not be related to the liver lipid

content (passive distribution), which indicates that active enrichment could be more likely. Passive accumulation might be more important for the higher brominated congeners, since these congeners can theoretically display a higher liver accumulation due to a higher log K_{ow} value in combination with their larger molecular size, which hinders their ability to cross membranes.[13] Higher brominated congeners also showed higher liver accumulation in this study (Figure 2). This observation was statistically significant for dab, plaice, and sole (R^2 = 0.94, 0.90, and 0.92 ($p>0.05$), respectively). For bib and whiting, a slight trend can be observed, but was not statistically significant. These observations might suggest that tissue dependent accumulation might well be related to animal species as to compound characteristics. Profile differences could also be observed between muscle and liver; BDE 47 contribution was significantly lower in liver than in muscle. No possible explanation could be found for this observation.

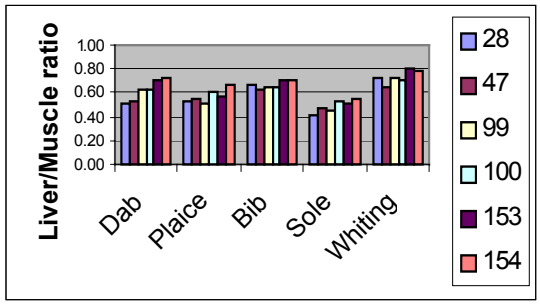

Figure 2. Liver accumulation ratio per congener in different fish species; ratios higher than 0.50 indicate preferential liver accumulation. Ratios are higher for higher degree of bromination.

Acknowledgments
The authors wish to thank the VLIZ (Flanders Marine Institute) for their logistic assistance.

References

1. de Boer, J.; de Boer, K.; Boon, J.P. In The Handbook of Environmental Chemistry, New Types of Persistent Halogenated Compounds; Paasivirta, J., Ed.; Springer-Verlag: 2000; pp 61-95, ISBN 3-540-6583-6.
2. BSEF website (accessed March 2003) Major Brominated flame retardants volume estimates. Bromine Science and Environmental Forum, Brussels, Belgium, 2003.
3. De Wit, C. Chemosphere 2002, 46, 583-624.
4. Hale, R.C.; La Guardia, M.J.; Harvey, E.P.; Matteson, M.; Duff, W.H.; Gaylor, M.O. Environ. Sci. Technol. 2001, 35, 4585-4591.
5. Darnerud, P.O.; Eriksen, G.S.; Jóhannesson, T.; Larsen, P.B.; Viluksela, M. Environ. Health Perspect. 2001, 109, 49-68.
6. Bergman, A. Organohalogen Compd. 2000, 47, 36-40.
7. Jacobs, M.N.; Covaci, A.; Schepens, P. Environ. Sci. Technol.2002, 36, 2797-2805.
8. Delhez, E.J.M.; Carabin, G. Estuar. Coast. Shelf. S. 2001, 53, 477-491.
9. de Boer, J.; Wester, P.G.; van der Horst, A.; Leonards, P.E.G. Environ. Pollut. 2003, 122, 63-74.
10. Boon, J.P.; Lewis, W.E.; Tjoen-A-Choy, M.R.; Allchin, C.R.; Law, R.J., de Boer, J.; Ten Hallers-Tjabbes, C.C.; Zegers, B.N. Environ. Sci. Technol. 2002, 36, 4025-4032.
11. Dodder, N.G.; Strandberg, B.; Hites, R.A. Environ. Sci. Technol. 2002, 36, 146-151.
12. Burreau, S.; Broman, D.; Örn, U. Chemosphere 2000, 40, 977-985.
13. Kierkegaard, A.; Balk, L.; Tjärnlund, U.; De Wit, C.; Jansson, B. Environ. Sci. Tecnol. 1999, 33, 1612-1617.