PBDEs in freshwater mussels and fish from Flanders, Belgium

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Introduction

Polybrominated diphenylethers (PBDEs), a class of brominated flame retardants (BFRs), are widely used in textiles, plastics, electronic equipment and other materials for more than 30 years[1]. Due to their massive use, PBDEs have become ubiquitously present in aquatic organisms and it was recently evidenced that their levels seem to increase rapidly[1]. Higher PBDE concentrations were found in biota from freshwater compared to similar marine species[2]. This is probably due to a higher pollution load found near point pollution sources that are almost exclusively inland located.

Zebra mussels (*Dreissena polymorpha)* fulfil the requirements of a good biomonitoring organism for freshwater ecosystems: they are easy to collect and to handle, are available in sufficient numbers, have a relative long lifespan, are sedentary and resistant to various types of pollution without suffering a too high mortality and have a high filtration rate which favours the bioaccumulation of organic contaminants[3]. Fish species are another suitable tool for the biomonitoring of organic contaminants. The occurrence of PBDEs in fish species from Europe has already received some attention[4,5], but the amount of data is still limited.

The aim of this study was to evaluate the occurence of PBDEs in zebra mussels and several representative freshwater fish species (eel, carp and gibel carp) at different sites in Flanders, Belgium. In parallel, other organohalogenated contaminants, such as polychlorinated biphenyls (PCBs), *p,p'*-DDE and hexachlorobenzene (HCB) were also measured and their relationship with PBDEs was investigated.

Methods and materials

Study area and sample processing

Thirteen sites including canals (7 sites), lakes (5 sites) and 1 pond, where indigenous zebra mussel are present in sufficient number, were selected in Flanders (Belgium). From previous sediment quality evaluation[6], it was shown that the sites covered a wide range of pollution levels. Mussels were collected in September 2002, the soft body parts of the mussels were removed from the shell and byssus threats were removed. Each sample consisted of a pooled sample of 25 individual zebra mussels which were homogenised using an Ultra-Turrax T8 homogeniser. Samples were stored at -20°C until analysed.

In September and October 2002, juvenile eels (*Anguilla anguilla*) were captured at 4 locations (1 canal, 1 pond and 2 basins), while carp (*Cyprinus carpio*) and gibel carp (*Carassius auratus gibel carpio)* were captured at 2 canals and 1 pond and at 1 basin and 2 canals, respectively. The muscle or liver tissues were dissected and stored at –20°C until analysis.

Extraction and analysis

The following PBDE congeners 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183 were investigated, while *p,p'*-DDE, hexachlorobenzene (HCB) and PCB congeners 28, 31, 74, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 153, 156, 163, 170, 180, 183, 187, 194 and 199 were also measured. The method used for sample preparation and analysis was previously described in detail[7,8]**.** Briefly, the available amount of tissue (50-500 mg for fish liver and 1-4 g for mussels and fish muscle) was ground with Na_2SO_4, internal standards were added and the mixture was extracted for 2 h with 75 ml hexane:acetone (3:1, *v/v*) into a hot Soxhlet manifold. After concentration, the extract was subjected to clean-up on acidified silica and analytes were eluted with 15 ml n-hexane followed by 10 ml dichloromethane. The eluate was concentrated to 80 µl and transferred to an injection vial.

PBDEs were determined on an Agilent GC/MS operated in electron-capture negative ionisation and equipped with a 25m x 0.22mm x 0.25µm HT-8 capillary column. PCBs and OCPs were determined on an Agilent GC-µECD equipped with a 50m x 0.22mm x 0.25µm HT-8 capillary column. Recoveries of target compounds ranged between 75 and 90%.

In mussels and fish muscle, method limits of quantification (LOQ) for individual BDE congeners ranged between 0.01 and 0.02 ng/g wet weight (ww). For individual PCB congeners, LOQ ranged between 0.03 and 0.16 ng/g ww, while for HCB and *p,p'*-DDE, LOQ were 0.23 and 0.63 ng/g ww, respectively. In

fish liver, LOQ for PBDEs, they were 0.1 ng/g ww, while for individual PCB congeners ranged between 0.5 and 1 ng/g ww. QC was done as described by Voorspoels et al.[8]

Results and discussion

PBDE concentrations in mussels

The average concentrations of PBDEs, PCBs and selected OCPs in zebra mussel at the different sites are given in Table 1. At most sites, individual PBDE congeners were present at detectable levels in mussel tissue, with the mean ∑ PBDE concentration ranging from 0.15 to 1.82 ng/g ww. Congeners 28 and 66 were below LOQ at most sites. Highest levels of PBDEs were measured in zebra mussels from Zennegat and Zuid-Willemsvaart canal, while the lowest levels were measured in zebra mussels from D-Schoten. The sampled sites covered a broad range of concentration of ∑ PCB in mussels going from 6.2 to 102 ng/g ww (Table 1). HCB and *p,p'*-DDE could be measured in mussels from most sites, mean values ranging from nd (not detected) to 0.58 ng/g ww and from 0.66 to 6.5 ng/g ww, respectively. Analysis of pollutants in the mussels showed that the selected sites covered a wide range of pollution from background to high contamination.

Christensen and Platz[9] have observed that PBDEs concentrations in blue mussels collected from several marine and freshwater Danish locations (n=15) had a relatively low variation (range 0.08 - 0.22 ng/g ww) except one marine sample with a concentration of 0.81 ng/g ww. Values from the Danish blue mussels are in the same range or lower than PBDE levels measured in freshwater mussels from Flanders. Zebra mussels samples collected from different locations in the Netherlands[10] showed variable concentrations of PBDEs. The lowest concentrations were measured in mussels from IJsel Lake (< 0.10 ng/g ww), while the highest were measured in mussels from Meuse (0.60 ng/g ww) and Dommel (1.05 ng/g ww). The PBDE profile is similar with the profile measured in the present study in mussels from Flemish locations. Mussels collected from Greenland[11] contained much lower PBDE levels (the highest measured value was 0.11 ng/g ww). PBDE concentrations in zebra mussels collected from different sites of Stockholm (Sweden)[12] ranged from 0.4 to 0.79 ng/g ww. The congener pattern was dominated by BDE 47 and BDE 99 and was similar to the penta-BDE technical product.

Recently, it was shown that for PBDEs and PCBs, no significant differences could be found between indigenous and transplanted mussels[3]. For

organic contaminants significant correlations between pollutant levels in transplanted/caged and resident mussels were found with R^2 values up to 0.98. Booij et al.[13] compared concentrations of PCBs and PBDEs in blue mussel (*Mytilus edulis*) between indigenous and transplanted mussels at one site in the North Sea. Although mussels were exposed for 42 days, concentrations in transplanted mussels were up to 2 and 10 times lower than in indigenous mussels for PCBs and PBDEs, respectively. The equilibrium time for PBDEs seemed to be longer than for PCBs as already observed by Gustafsson et al[14].

PBDE levels in fish species

The average concentrations of PBDEs, PCBs and selected OCPs in eel liver, carp and gibel carp muscle at the different sites are given in Table 1. BDE 66 and 85 were not quantified in any fish sample, while BDE 49 was not quantified in eel. Except for one site (Blokkersdijk) where PBDEs were below LOQ in carp muscle, fish samples from all other sites contained detectable PBDE levels (Table 1). The highest PBDE concentrations (14.0 ± 14.1 ng/g ww) were measured in eel liver from Watersportbaan (Ghent). The sampled sites covered a broad concentration range of PCBs and OCPs, with the highest values being consistently measured in eel liver.

PBDE concentrations in eel liver from the river Elbe (Germany) and its tributaries ranged from 1.5 to 7.7 ng/g ww in Elbe near the Czech border and from 2.2 to 5.8 ng/g ww in the Elbe tributary[4]. The measured PBDE profile was similar to that described in the present study. BDE 47 was the main congener contributing with 60-80% to the total PBDEs (mean 69%), followed by BDE 100 and by congener BDE 99, 153 and 154 at much lower percentages.

Eel samples collected from 18 different locations in Flanders[15] showed a great variation in ΣPBDE concentrations between 10 and 6000 ng/g ww. The highest concentrations were measured in eel from the Scheldt and its main tributary, the Leie. It is known that the area covered by the two rivers is subjected to industrial activities using BFRs (e.g. textile factories). The same profiles as in our eel samples were once more observed.

Allchin and Morris[16] determined PBDEs in edible fish (brown trout *Salmo trutta* and eel *Anguilla anguilla*) from the rivers Skerne and Tees in North East UK, downstream of a BFR manufacturing plant. PBDEs were detected in fish from all sites, and the lowest concentrations were found in the two upstream Tees sites. Mean ΣPBDE concentrations in trout at these sites were 4.9 and 5.3 ng/g ww, respectively. The highest concentrations (mean and maximum ΣPBDE

concentrations 118 and 197 ng/g ww) were observed just downstream of the plant in which PBDE formulations had previously been manufactured[5], and declined with downstream with the distance to the plant. Mean ΣPBDE concentrations in eel muscle sampled at 4 sites further downstream in the river Tees as far as the tidal barrage ranged from 130 to 235 ng/g ww.

Carp from Detroit river (US) contained similar PBDE levels as those found in carp and gibel carp from Flanders (Belgium), while carp samples collected from a polluted area of Des Plaines river contained higher PBDE concentrations (up to 18 ng/g ww). In addition, the PBDE profile in the latest samples was not similar with carp samples from Flanders and Detroit river which pointed to additional sources of octa- and deca-BDE formulations.

PBDE profiles in freshwater biota.

The contribution of each PBDE congener to the ΣPBDEs in each investigated aquatic species from Flanders is presented in Figure 1. As already reported in literature[9,10,13], BDE 47 and BDE 99 were the dominant congeners in mussels, followed by BDE 100. Other congeners had a lower contribution and the mussel profile closely reflects the penta-BDE technical product. This is in accordance with the low metabolic capabilities of mussels and to their primary filtration function. BDE 183, a marker of the octa-BDE formulation, could also be found at low concentrations, but this can be attributed to the fine sediment particles present in the digestive tract of the mussels.

Contrarily, the PBDE profile was similar in all fish species, but different from the profile in mussels (Figure 1). BDE 47 was the dominant congener in all fish species (63-82%) followed by BDE 100. BDE 183 was not detected in any fish samples which is in accordance with previous observations from the Scheldt river (Belgium)[7]. BDE 99 was present in all fish species at very low concentrations or was not detected (carp). A significant debromination of BDE 99 and BDE 183 in the intestinal tract of carp has been already reported[17] and a conversion of BDE 99 to BDE 47, and of BDE 183 to BDE 154 and another (as yet unidentified) hexa-BDE congener was suggested. In another experiment, Stapleton et al.[18] demonstrated that, following a feeding experiment with BDE 209, at least 7 penta- to octa-BDEs were formed during 60 days of exposure. These congeners included BDE 153 and BDE 154, compounds which are commonly found in fish tissues and which are usually ascribed to exposure to the penta-mix PBDE formulation.

Low correlation coefficients ($R^2 < 0.35$) could be calculated between PBDE and PCB concentrations for each species or for all species taken together.

This indicates separate pollution sources with PBDEs and PCBs, with PBDEs expected to derive from point sources.

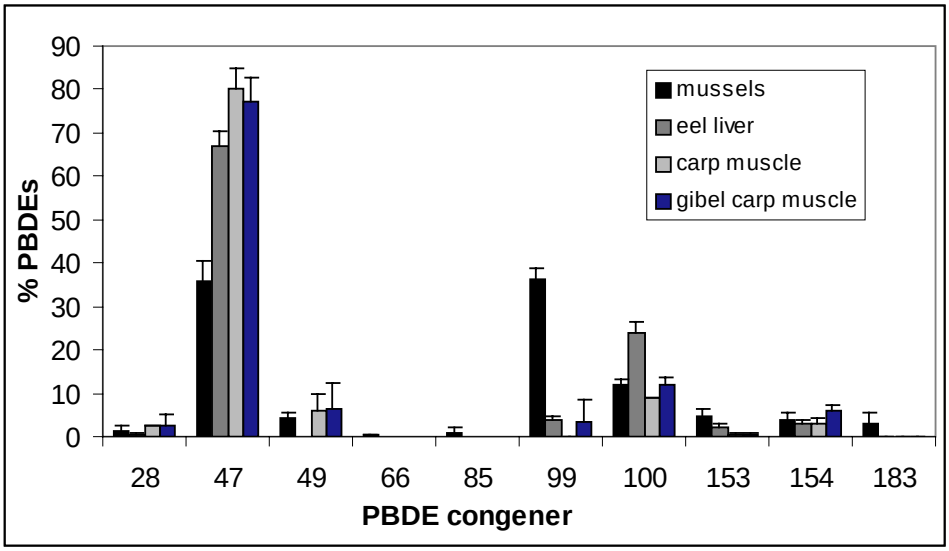

Figure 1. PBDE congener distribution (mean percentage ± 2SE) in mussels, carp, gibel carp and eel.

In conclusion, PBDEs were detected in all freshwater biota samples from Flanders. The measured concentrations had a large variation and this may be attributed to different industrial activities along the sampling sites. Compared to literature levels in zebra mussels were relatively high, whereas levels in fish tissues were moderately.

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References

1. de Wit C.A. (2002) *Chemosphere* 46, 583-624.
2. Hendriks A.J., Pieters H. and de Boer J. (1998) *Environ. Toxicol. Chem.* 17, 1885-1898.
3. Bervoets L., Voets J., Chu S.G., Covaci A., Schepens P. and Blust R. (2004) *Environ. Toxicol. Chem.* in press.
4. Lepom P., Karasyova T. and Sawal G. (2002) *Organohalogen Compounds* 58, 209-212.

5. Allchin C.R., Law R.J. and Morris S. (1999) *Environ. Pollut.* 105, 197-207.
6. De Cooman W., Florus M. and Devroede M.P. (1998) Characterisation of sediments of Flemish water course. Report Ministry of the Flemish Government, AMINAL, Brussels. p. 56 (in Dutch).
7. Jacobs M., Covaci A. and Schepens P. (2002) *Environ. Sci. Technol.* 36, 2797-2805.
8. Voorspoels S., Covaci A. and Schepens P. (2003) *Environ. Sci. Technol.* 37, 4348-4357.
9. Christensen J.H. and Platz J. (2001) *J. Environ. Monit.* 3, 543-547.
10. de Boer J., Wester P.G., van der Horst A. and Leonards P.E.G. (2003) *Environ. Pollut.* 122, 63-74.
11. Christensen J.H., Glasius M., Pecseli M., Platz J. and Pritzl G. (2002) *Chemosphere* 47, 631-638.
12. Lithner G., Holm K. and Ekström C. (2003) Metaller och organiska miljögifter i vattenlevande organismer och deras miljö i Stockholm 2001. ITM Rapport 108, 87 pp., Institute of Applied Environmental Research (ITM), Stockholm University, Sweden.
13. Booij K., Zegers B.N. and Boon J.P. (2002) *Chemosphere* 46, 683-688.
14. Gustafsson K., Bjork M., Burreau S. and Gilek M. (1999) *Environ. Toxicol. Chem.* 18, 1218-1224.
15. de Boer J., Allchin C., Zegers B., Boon J.P., Bransdma S.H., Morris S., Kruijt A.W., van der Veen I., van Hesselingen J.M. and Haftka J.J.H. RIVO rapport nr. C033/02, September 2002.
16. Allchin C.R. and Morris S. (2003) *Organohalogenated Compounds* 61, 41-44.
17. Stapleton H.M., Letcher R.J., Baker J.E. (2004) *Environ. Sci. Technol.* 38, 1054-1061.
18. Stapleton H.M., Alaee M., Letcher R.J. and Baker J.E. (2004) *Environ. Sci. Technol.* 38, 112-119.

Table 1. Mean concentrations (SD) of organic contaminants (ng/g wet wt) in zebra mussels, carp and gibel carp muscle and eel liver from lakes and canals in Flanders, Belgium.

Location	N	Lipids (%)	ΣPBDEs	% BDE 47	ΣPCBs	HCB	p,p'-DDE
Mussels							
AWW (Duffel)	3	1.3	0.30 (0.03)	31	20 (1)	ND	ND
Weerde (Zemst)	3	0.9	0.46 (0.07)	31	67 (5)	0.25 (0.02)	2.2 (0.4)
Nekker (Mechelen)	3	0.9	0.22 (0.04)	45	12 (1)	ND	1.1 (0.1)
Walenhoek (Niel)	3	1.6	0.26 (0.20)	35	7 (1)	ND	1.5 (0.1)
E10 (Schoten)	3	0.9	1.21 (0.26)	31	6 (1)	ND	0.5 (0.3)
Nete Canal (Nijlen)	2	0.5	0.48 (0.05)	22	45 (5)	0.34 (0.07)	0.7 (0.1)
Z-W Canal (Rekem/Lanaken)	4	0.8	1.82 (0.20)	36	50 (2)	0.58 (0.08)	1.4 (0.1)
H-Boch 1 (Kaulille)	2	0.8	0.35 (0.10)	43	46 (2)	0.47 (0.03)	1.3 (0.1)
H-Boch 2 (Dessel)	3	0.7	0.64 (0.14)	38	49 (5)	0.45 (0.04)	1.0 (0.2)
Beverlo (Lommel)	3	0.8	0.92 (0.21)	36	48 (6)	0.48 (0.01)	1.1 (0.1)
D-Schoten (Turnhout)	3	0.5	0.15 (0.04)	53	30 (2)	0.25 (0.01)	0.9 (0.1)
Mol-Dessel (Mol)	3	1.0	0.75 (0.03)	33	52 (1)	0.34 (0.04)	1.1 (1.1)
Zennegat (Walem)	3	1.4	1.48 (0.04)	36	102 (3)	ND	6.6 (0.5)
Eel							
Canal Ieper-Ijzer (Boezinge)	9	n.a.	3.64 (2.40)	72	311 (152)	2.24 (0.78	24.2 (37.4)
Oude Maas (Dilsen-Stokkem)	10	n.a.	2.46 (1.37	63	494 (329)	0.81 (0.37)	6.0 (5.0)
Zuun (Sint-Pieters-Leeuw)	11	n.a.	1.98 (1.08)	63	138 (95)	0.91 (0.22)	7.3 (2.9)
Watersportbaan (Ghent)	10	n.a.	14.0 (14.1)	72	393 (203)	0.71 (0.35)	11.0 (3.8)
Carp							
Canal Ieper-Ijzer (Boezinge)	7	1.3	1.56 (0.87)	83	74 (52)	0.61 (0.42)	9.4 (8.7)
Blokkersdijk (Antwerp)	10	0.6	< 0.10	n.a.	37 (6)	< 0.23	2.0 (0.4)
Durme (Hamme)	6	2.4	6.0 (3.26)	78	52 (17)	0.28 (0.07)	40.7 (13.0)
Gibel carp							
Zuun (Sint-Pieters-Leeuw)	8	1.3	0.62 (0.31)	73	25 (15)	0.44 (0.17)	5.9 (2.6)
Canal Willebroek (Willebroek)	4	0.9	3.75 (0.68)	76	210 (43.)	0.55 (0.16)	7.9 (2.2)
Scheppelijke Nete (Balen)	5	0.4	0.97 (0.48)	82	132 (82)	0.33 (0.05)	9.5 (9.5)

n.a. – not available