PBDEs AND HBCDs IN FLEMISH EELS: LEVELS AND ISOMERIC PATTERNS

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Abstract
Isomer-specific distribution of PBDEs and HBCDs was investigated in 50 pooled eel muscle samples collected throughout Flanders, Belgium. Concentrations of Σtri-hepta PBDEs ranged between 4 – 5811 ng/g lipid weight (lw) with a median value of 79 ng/g lw. One outlier (5811 ng/g lw) was identified, although the remaining samples did not follow a Gaussian distribution. The majority of samples was characterised by low tri-hepta levels up to 200 ng/g lw, whereas three samples contained levels between 200 – 1200 ng/g lw. These samples were collected near highly industrialized areas in Flanders. BDE 47 was the dominant PBDE congener in the samples in which BDE 209 could not be detected (n = 44). However, when BDE 209 was detected (n=6), it was the most dominant PBDE congener, though at low levels varying between 10 – 87 ng/g lw. Levels and profiles of PBDEs in the most contaminated eel samples were compared with those measured in sediment samples from the same locations, measured in a previous study. Sediment samples were characterised by low Σtri-hepta PBDEs concentrations (2 – 65 ng/g dw) and high BDE 209 levels (20 - 2400 ng/g dw). The PBDE profile in sediment is different compared to the eel profile, with BDE 209 being the dominant PBDE congener in all sediment samples. Differences in bioavailability and bioaccumulation potential of PBDE congeners explain the different profiles observed in eels and sediment. ΣHBCDs in eels ranged between 14 – 4397 ng/g lw with median value of 73 ng/g lw. One outlier of 4397 ng/g lw could be detected. The majority of the eel samples (34/50) had ΣHBCD levels < 100 ng/g lw, while 5 eel samples were heavily contaminated, with HBCDs levels ranging between 1000 and 4400 ng/g lw. The α-HBCD isomer was the dominant isomer (mean 84%) in all samples, followed by γ-HBCD and β-HBCD.

Introduction
Brominated flame retardants (BFRs) have been extensively used in materials such as plastics, textiles, furnishing foam, and electronic circuit boards[1] to reduce the risk of fire and meet fire safety regulations [2]. Additive BFRs, such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), are more likely to be released into the environment compared to reactive BFRs and they tend to be stable and persist in terrestrial and aquatic environments. Due to their high hydrophobicity, these chemicals are often associated with soils and sediments. However, they have been found to accumulate in living organisms and biomagnify throughout terrestrial and aquatic food chains[3,4].
The aims of the present study were firstly to investigate the PBDE and HBCD distribution in eels throughout the freshwater system in Flanders and secondly, to compare the isomeric profiles of PBDEs and HBCDs in eel and sediment from the most polluted areas. European eel (*Anguilla anguilla* L.) in its yellow eel stage is chosen as bio-indicator for the monitoring of environmental contaminants as this stage is characterised by sedentary behaviour[5]. Eel analysis gives a representative description of contamination patterns within 300 m where it was caught. Furthermore, eel is a fatty fish species (~15% lipids in the muscle), assuring an optimal accumulation of lipophilic contaminants, such as BFRs[6]. The present study expands current knowledge regarding the PBDE and HBCD concentrations, their patterns and distribution profiles in the freshwater system of Flanders.

Materials and methods
Samples. Eels were collected in 2008 from 50 various locations throughout Flanders, Belgium. Between 3 – 10 eels were caught per location, their muscle was pooled and further analysed for PBDE congeners (28, 47, 49, 66, 99, 100, 154, 153, 183 and 209) and for HBCD (α, β and γ) isomers.

Sample preparation. Eel muscle samples were spiked with internal standards (BDE77, BDE 128, ^{13}C-BDE 209 and ^{13}C-HBCDs), hot Soxhlet extracted (2h) with hexane:acetone (3:1) and cleaned-up on acidified silica[7]. Minor adaptations were required as PBDEs were analysed by GC-ECNI/MS and HBCDs by LC-MS/MS. The cleaned extract was evaporated to dryness, redissolved in 0.5 ml hexane and eluted from pre-packed silica cartridges with 6 ml hexane (for GC analysis) and 6 ml DCM (for LC analysis). Both fractions were evaporated to dryness and redissolved in 100 µl iso-octane or methanol, respectively.

GC analysis. The determination of PBDEs was performed with an Agilent 6890GC-5973MS equipped with a 15 m x 0.25 mm x 0.10 µm DB-5 capillary column and operated in electron capture negative ionisation (ECNI) mode. The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. Helium was used as carrier gas at constant flow (1.0 mL/min) and with methane as moderating gas. The MS was operated in SIM mode (*m/z* 79 and 81 were monitored for the entire run, *m/z* 487 and 495 were monitored for BDE-209 and ^{13}C-BDE 209, respectively). Dwell times were set to 40 ms. One µl of the extract was injected in solvent vent mode and the splitless time was 1.50 min. The temperature of the DB-5 column was programmed from 90°C, kept for 1.5 min, then increased with 15°C/min to 295°C, kept for 15 min.

LC analysis. Determination of ΣHBCDs and separation of α-, β-, and γ- HBCD was achieved using a dual pump Agilent 1100 Series liquid chromatograph equipped with autosampler and vacuum degasser. An Agilent Zorbax Extended-C_{18} reversed phase analytical column (50 mm x 2.1 mm i.d., 3.5 µm particle size) was used. A mobile phase of (a) water and (b) methanol at a flow rate of 200 µL/min was applied for elution of HBCD isomers; starting at 75% (b) then increased linearly to 100% (b) over 7 min; this was held for 12 min followed by a linear decrease to 75% (b) over 0.5 min and held for 10 min. The target analytes were baseline separated on the reversed phase C_{18} column with retention times of 7.0, 7.5, 7.8 min for α-, β- and γ- HBCD respectively. Mass spectrometric analysis was performed using an Agilent 6410 triple quadrupole mass spectrometer operated in the ES negative ion mode. MS/MS detection operated in the MRM mode was used for quantitative determination of the HBCD isomers based on m/z 640.6→ m/z 79 and m/z 652.6→ m/z 79 for the native and ^{13}C-labelled diastereomers, respectively.

QA/QC. The analytical procedures were validated through analysis of procedural blanks, duplicate samples, and certified material SRM 1945 (PBDEs in whale blubber, which has also indicative values for HBCDs). Obtained values were not deviating with more than 10% from the certified values and all samples were blank-corrected. Method quantification limits (LOQs) for individual PBDE congeners and individual HBCD diastereomers were based on procedural blanks (3x SD) and the amount of sample taken for analysis (typically 1 g eel muscle). LOQs for tri-hepta PBDEs range between 1-2 ng/g lipid weight (lw), for BDE 209 LOQ was 10 ng/g lw, while LOQs were 1, 2 and 2 ng/g lw for α-, β- and γ-HBCD, respectively. Samples with concentrations below LOQ were calculated as f*LOQ with f being the fraction of samples above LOQ.

Results and discussion

PBDEs. In total 50 pooled eel muscle tissue samples were analysed and lipid percentage ranged between 2-21%. Σtri-hepta BDEs ranged between 4 – 5811 ng/g lw with a median value of 79 ng/g lw. One highly contaminated sample of 5811 ng/g lw could be identified. The majority was characterised by low tri-hepta levels up to 200 ng/g lw, whereas three samples contained levels between 200 – 1200 ng/g lw. These samples were caught near a highly industrialized area in Flanders, Oudenaarde. This area is known for its textile industry and has been the subject of regular research regarding BFRs. Eel collected from this region in 2000 contained a maximum sum of tri-hepta BDEs of 26500 ng/g lw, samples collected in 2006 were still highly contaminated compared to other regions in Flanders, but PBDE levels declined to 700 – 1000 ng/g lw[8]. A stabilizing effect has been seen in recent years as current samples showed concentrations between 200 – 1200 ng/g lw. The outlying eel sample originated from a location ~50 km downstream of Oudenaarde, also located on the river Scheldt, but closer its mouth.

Similar to Allchin and Morris[9], who detected large amounts of PBDEs in eel living downstream from the Newton Aycliffe sewage treatment works, current results indicate the large impact of usage of chemicals on the nearby environment and the importance of monitoring and legislation as levels declined from 2000 to 2006. The majority of our eel samples contain similar Σtri-hepta BDE concentrations compared to other European studies

(Table 1). Bragigand et al.[10] analysed eel originating from two rivers Seine and Loire with a concentration range between 26 – 108 ng/g lw. Van Leeuwen et al.[11] recently analysed eel samples to estimate their contribution to human PBDE intake. The levels of Σtri-hepta PBDE congeners ranged between 3 – 3139 ng/g lw with a median value of 261 ng/g lw indicating the presence of some highly contaminated eel samples with concentrations between 1000 – 3000 ng/g lw, but the majority of the samples contained a median value of 200 ng/g lw, similar to our samples. No conclusive explanation was given for these higher concentrations although sampling of larger, more industrialized waters is thought to lead to more contaminated biota[11].

Table 1. Overview of Σtri-hepta PBDE congeners and ΣHBCDs in European and US studies, including results of the current study.

Σtri-hepta PBDEs	**Median**	**Range**	**Reference**
France[a]	/	26 – 108	10
The Netherlands[a]	261	3 – 3139	11
USA[b]	/	10 - 5652	12
Belgium[a]	/	660 - 1010	8
Belgium[a]	**79**	**4 - 5811**	***present study***
ΣHBCDs			
The Netherlands	/	6 - 690	11
Belgium	/	< 1 - 266	22
Belgium	/	2600 - 10100	8
Belgium	**73**	**14 - 4397**	***Present study***

[a] - BDE 28, 47, 49, 66, 100, 99, 153, 154 and 183
[b] - BDE 17, 25, 28+33, 30 47, 49, 66, 71, 75, 85+155, 99, 100, 116, 119,138, 153, 154, 156, 181, 183, 190, 191, 203, 205, 206 and 209.

In this study, BDE 47 was the dominant congeners in most samples (n = 44) and accounted on average 60% of Σtri-hepta PBDEs. BDE 100 accounted for 19%, followed by BDE 99 for 5%. BDE 209 could only be quantified in 6 samples with concentrations ranging from 10 up to 87 ng/g lw. Despite its low detection frequency, BDE 209 is the dominant congener in all samples where it was detected. Presence of BDE 209 in eel muscle tissue is not correlated with high Σtri-hepta PBDE congeners, indicating a different source of origin. This pattern has been reported in several studies involving aquatic biota[8,12] and is indicative of former use of the Penta-BDE mixture[13], combined with low bioavailability of BDE 209[14].

Ashley et al.[12] reported on similar PBDE profiles in American eel samples. High BDE 47 and low BDE 99 levels were suggested as a possible outcome of metabolic pathways, present in eels. Such pathways have been described for carp (*Cyprinus carpio carpio* L.)[15]. According to this statement, the BDE 47/BDE 99 ratio gives an estimate of the time of exposure. High ratios are indicative of past exposure as debromination has occurred, low ratios are indicative of recent exposure as debromination still has to occur. Calculating BDE 47/BDE 99 ratios for our eel samples, two groups can be distinguished. The first group contains nearly all eel samples (n=48) and are characterised by high BDE 47 levels and low BDE 99 level (< 17 ng/g lw), only two samples, which represent the second group, contain significant higher BDE 99 levels (40 and 260 ng/g lw). This finding suggests recent exposure of both locations which is supported by the highly industrialized surroundings.

PBDE results were obtained by courtesy of VMM[16] for sediment samples collected at the three locations which correspond to eel samples containing highest PBDE levels. PBDE profiles in eel and sediment samples were compared but no obvious associations between eel and sediment from the same location could not be found. Overall, sediment samples were characterised by low Σtri-hepta PBDEs concentrations between < 2 – 65 ng/g dw, but higher BDE 209 levels ranging from 20 up to 2400 ng/g dw. This shift in the PBDE congener profile compared to eel is due to different bioavailability and bioaccumulation potentials between PBDE congeners. Due to its structural formation and high molecular weight, BDE 209 is expected to be less bioavailable compared to other, smaller congeners, such as BDE 47. However, Eljarrat et al.[17] described bioavailability of higher BFRs, such as BDE 209, but only in highly contaminated surroundings where BDE 209 sediment concentrations ranged

up to 12 µg/g dw, an order of magnitude higher than our most contaminated sample. Under these conditions, BDE 209 was found in 14 out of 15 fish muscle tissue samples with concentrations up to 700 ng/g lw, one order of magnitude higher compared to our most contaminated eel sample. Apparently, presence of BDE 209 in Belgian sediment samples is not high enough to efficiently transfer to biota.

HBCDs. ΣHBCDs in eel ranged between 14 – 4397 ng/g lw with a median value of 73 ng/g lw. One highly contaminated sample of 4397 ng/g lw could be detected. 34 out of 50 eel samples contained ΣHBCDs levels <100 ng/g lw, eleven samples were situated between 400-900 ng/g lw and five were heavily contaminated with HBCD levels between 1000 and 4400 ng/g lw. α-HBCD was the dominant isomer in all samples (average 85%), followed by γ-HBCD (10%) and β-HBCD (6%). Although the HBCD technical mixture is mainly composed of γ-HBCD, several mechanisms have been suggested to explain the isomeric shift to α-HBCD, which is the most common isomer in biota. Several mechanisms have been suggested. Higher solubility of the α-HBCD isomer (49 µg/l) compared to γ-HBCD (2 µg/l) might cause a preferential transfer of α-HBCD towards the aquatic environment and might explain the higher exposure of aquatic organisms to this isomer compared to γ-HBCD[18]. Metabolic elimination of β-HBCD and γ-HBCD or preferential accumulation of the α-HBCD isomer could also be possible[19]. Recently, reports have been made on photolytic degradation of γ-HBCD in dust, explaining higher α-HBCD levels[20]. Heeb et al.[21] elucidated kinetics involved with this isomeric shift which can be induced by thermal treatment. Therefore, industrial discharge of textile and plastic industries might be mainly composed of α-HBCD as HBCD is incorporated in the products by heating[22].

Roosens et al.[8] analysed eel samples in the region of Oudenaarde with levels between 2600 – 10100 ng/g lw. Although PBDE levels stagnated between 2006-2009, HBCD levels have declined substantially. Similar to PBDEs, the presence of nearby industries largely affect levels of BFRs in surrounding biota. Morris[22], for example, analysed fish from the river Scheldt but its reported range was merely between <1 – 266 ng/g lw with an average 20 times lower compared to the current study. Identical research in the Netherlands led to ΣHBCD levels between 6 – 690 ng/g lw[11] (Table 1). ΣHBCDs in corresponding sediment samples were all below limit of detection.

PBDEs versus HBCDs. A large concentration range is covered for both compounds throughout Flanders, in a sampling grid used in the last 15 years for the monitoring of PCBs and pesticides[6]. HBCD levels were higher compared to detected PBDE levels in all samples. One highly contaminated sample was detected for Σtri-hepta PBDEs and one for ΣHBCDs. Although samples with high PBDE content typically contained higher levels of HBCDs, the opposite was not always true which indicates a different usage of both BFRs (Figure 1).

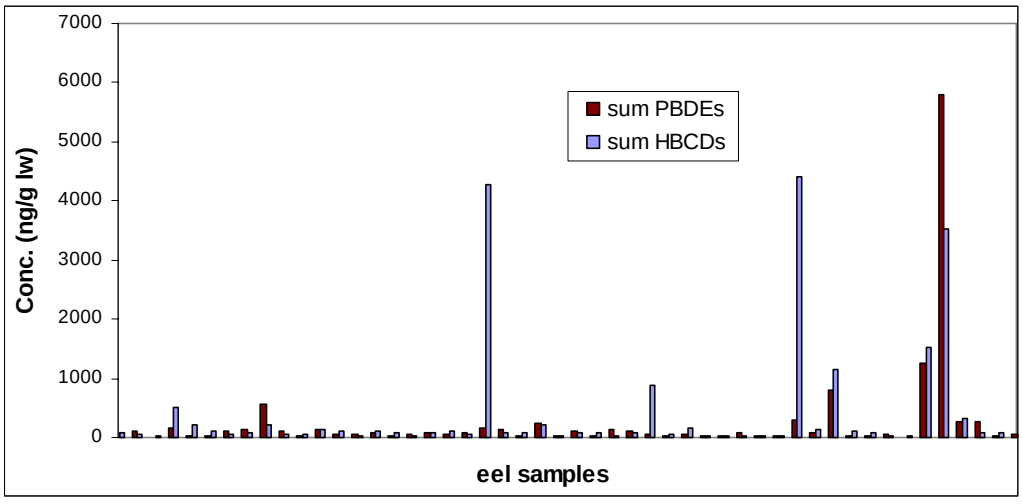

Figure 1. ΣPBDEs and ΣHBCDs in 50 eel samples from Flanders, Belgium

Compared to the literature, HBCD and PBDE concentrations in eel are similar with other European studies, discarding the highly contaminated samples. As the current study proves large variability in BFR concentration, even on the same river, comparison between studies depends largely on the amount of samples analysed and the sampling location. Although not reported here, the concentration of PCBs remains high in Flanders, with only 28% of the sampling locations below the Flemish consumption norm of 75 ng/g ww for the 7 marker PCBs.

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