Distribution of Hexabromocyclododecane Diastereomers in Marine Biota in the Western Scheldt Estuary

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Introduction

Hexabromocyclododecane (HBCD) is one of the most common additive flame retardant mainly used in polystyrene foams with the global market consumption in 2001 at about 16 700 tons.[1] HBCD production results in a technical product consisting mostly of three diastereomers, α-, β-, and γ-HBCD (Figure 1), with the γ–isomer being the predominant one.

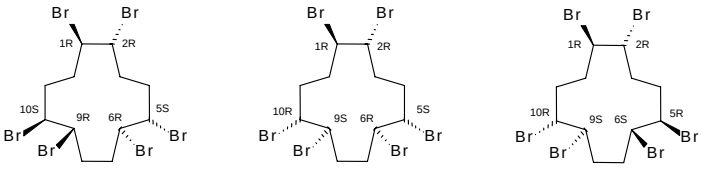

Figure 1. Structures of the HBCD diastereomers.

HBCD has a high bioaccumulation potential and bioavailability and has been found in increasing concentrations in environmental samples and in biota.[2,3] Diastereoisomer-specific analysis of HBCD was achieved by reversed phase HPLC[4] and consistently higher concentrations of the α-isomer compared to γ–isomer have recently been reported, while the β-isomer was non-detected in the majority of samples.[5,6]

The Western Scheldt Estuary is subjected to a variety of suspected brominated flame retardants (BFR) sources such as a BFR manufacturing plant (Terneuzen, The Netherlands), the Antwerp harbour and textile industry located further upstream the river. Recently, polybrominated diphenyl ethers (PBDEs) were investigated in marine species of different trophic levels collected from the Scheldt Estuary[7]. In Europe, HBCD is more widely used than PBDEs[1] and high levels of HBCD have already been reported in sediments from the Schedlt[8]. So far, there is very little known about differences in toxicity, bioavailability and bioacumulation of HBCD diastereoisomers. In this

paper, we report on the levels of the HBCD diastereomers in various marine species and sediment from the Western Scheldt.

Materials and Methods

The sampling of different marine species from the Western Scheldt Estuary has been previously described.[7] Fillet and liver of gadoids (whiting, *Merlangius merlangus*; bib, *Trisopterus luscus*) and flatfish (sole, *Solea solea*; plaice, *Pleuronectus platessa*) were sampled and pooled. Pools consisted of 3-6 individuals. Eel muscle and whole shrimps from the same locations were also sampled. Sample preparation has been described elsewhere.[7]

LC/MS-MS was performed using a ThermoFinnigan TSQ Quantum MS equipped with a Surveyor HPLC pump. The MS was used in the electrospray negative ion mode using Selective Reaction Monitoring (SRM) for $[M\text{-}H]^-$ (*m/z* 640.6) $\rightarrow Br^-$ (*m/z* 79.0 and 80.7). The collision energy was set at 17 and 21 eV for *m/z* 79.0 and 80.7, respectively.

Diastereomers were separated using Water's reverse phase column Symmetry^{TM} C_{18} (2.1mm x 150mm; 5µm) with mobile phase set to 60% H_2O/30% CH_3OH/10% ACN for 0.5 min followed by a linear gradient for 5 min to 50% ACN/50% CH_3OH which was held for 9 min. The flow rate was set at 0.25 mL/min. Injection volumes were 20 µL. Each extract was analysed in duplicate. Standard solutions of α-, β- and γ-HBCD in toluene were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

Results and Discussion

Separation of the three diastereomers of HBCD in standard mixture, sediment and bib liver samples, respectively using the reverse phase column is shown in Figure 2.

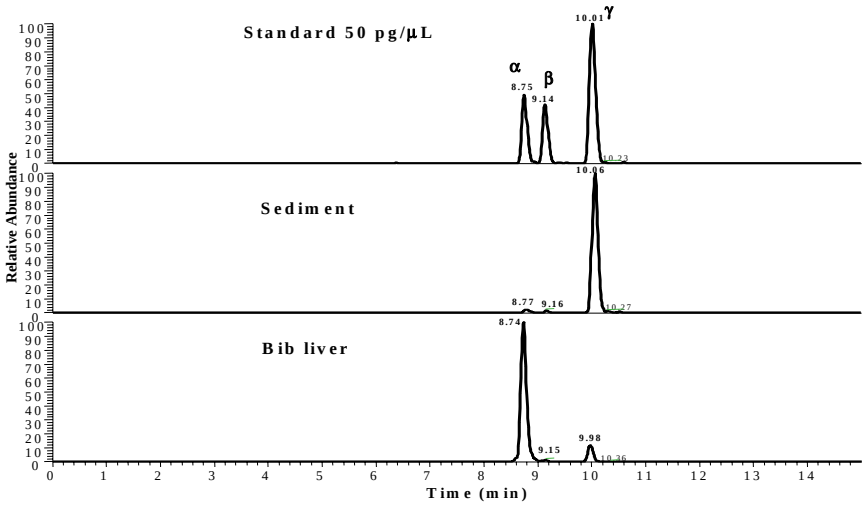

Figure 2. Reverse phase chromatographic separation of HBCD diastereomers in standard mixture, sediment and bib liver samples.

Table 1. Levels of α-HBCD and γ-HBCD in biota samples form Scheldt Estuary

	Shrimp		Eel		Sole				Plaice				Bib				Whiting			
Loc[1]	Whole		M[2]		M[2]		L[3]		M[2]		L[3]		M[2]		L[3]		M[2]		L[3]	
	α	γ	α	γ	α	γ	α	γ	α	γ	α	γ	α	γ	α	γ	α	γ	α	γ
13					133	6	153	< 1			23	4	97	39	73	5				
14					1096	13	679	< 1	38	< 2	26	6	53	43	153	5	75	38	168	9
15	28	18	27	3	357	17	110	8					53	< 1	102	10	45	< 1	16	< 2
16	38	< 2			114	11	99	< 1			21	8					65	51	244	35
17			7	2																

[1] No of location corresponding to reference 7. [2] muscle; [3] liver

Measurable values of α-HBCD have been found in all analysed samples, while γ-HBCD could be quantified in a much lower number of samples (Table 1). The β-HBCD isomer was present only in plaice and bib liver at levels below the limit of quantification (LOQ). The γ-HBCD isomer could not be detected in sole liver suggested that, while in sole there is a strong differentiation in HBCD isomers between muscle and liver tissue, much less differentiation probably occurs in plaice, bib and whiting.

Absolute levels of HBCD determined as the sum of α-HBCD and γ-HBCD diastereomers were in the range of 9 – 1109 ng/g lipid weight (lw), while the sum of PBDEs in these samples ranged from 71 - 2410 ng/g lw.[7] The total HBCD levels were confirmed by semi-quantitative GC analysis performed during the PBDE analysis, although it can not be ruled out that the present determination may have been affected by long time storage of the extracts (~ 1 year).

Published data on HBCD levels in biota are very scarce. The comparison between data produced by different studies is also hampered by different ways of expressing results (wet weight vs. lipid weight) as shown in **Table 2**. However, the large variation between the available data might report on high differences of HBCD levels in biota. In our study highest HBCD levels were measured in fish samples from location 14 situated in the vicinity of a HBCD producing plant.

Table 2. Literature data on HBCD and PBDE levels in fish.

Reference	**α-HBCD**	**γ-HBCD**	**Total**	**PBDE**
Present study	*7 - 1096* [a]	*< 1 - 51* [a]	*< 9 - 1109* [a]	*71 – 2410* [a]
Tomy G.T. *et al.*[5]	0.4 – 3.8[b]	0.1 – 0.8[b]	0.5 – 4.6[b]	not estimated
Gerecke A.C. *et al*[6]	210[a]	33[a]	25 – 210[a]	not estimated
Eljarrat E. *et al*[3]			nd – 1172[b]	nd – 446[b]
Allchin C.R. *et a*[8]			20.3 – 10300[b]	4.9 – 294[b]

[a] – lipid weight based b – wet weight based

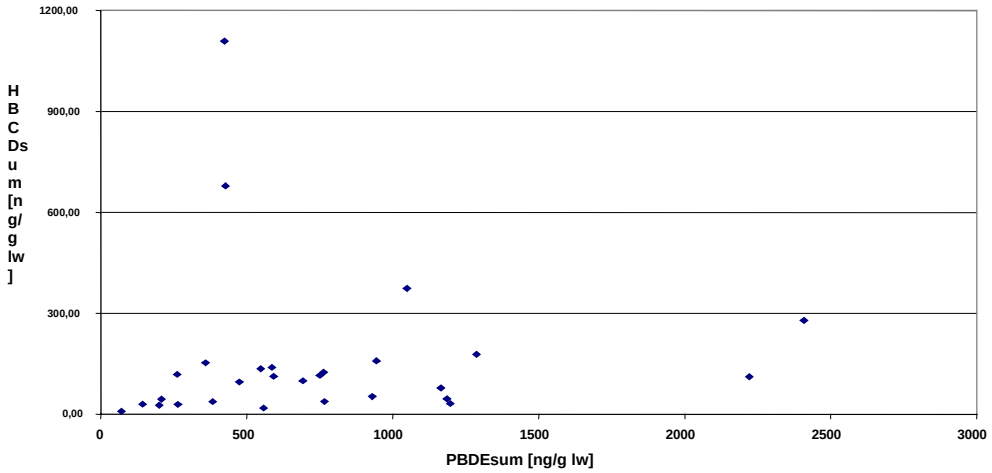

Figure 3. Correlation between total PBDE and HBCD values.

We have found that some species, such as bib and whiting, accumulated preferably α-HBCD in the liver compared to the level in muscle, while sole accumulated more α-HBCD in muscle (Table 3). The preferential accumulation of α-HBCD diastereoisomer in liver was calculated through the ratio between concentration of α-HBCD in liver and the sum of concentrations of α-HBCD in liver and muscle, all expressed in ng/g lipid weight.

$$R = \frac{C_{\alpha-L}}{C_{\alpha-L} + C_{\alpha-M}}$$

A value higher than 0.50 points to a preferential accumulation of the pollutant in the liver. Plaice samples were too limited in number in order to evaluate this species. Similar preferential liver accumulation in bib and whiting and preferential muscle accumulation in sole was already observed for PBDEs. [7]

Table 3. Ratio of α-HBCD diastereomers in fish species

Fish	Plaice		Sole		Bib		Whiting		Eel
Organ	L	M	L	M	L	M	L	M	M
α-L/(α-L+α-M)[a]	0.46*		0.41		0.68		0.72		
α-/γ–[b]	4.12	N.E.	469	34	18.1	1.9	13	1.6	6.63

[a] – ratio alpha in liver to (alpha in liver + alpha in muscle) from the same fish

[b] – ratio α-HBCD/γ-HBCD in particular organ

* - value based on 1 sample

Another interesting feature is ratio of α-HBCD to γ-HBCD in a particular organ. The ratio was mostly found higher for liver then for muscle with sole having much higher preference for α-HBCD (Table 3) in both tissues then any other investigated species. We have not found any literature data for diastereomer distribution in fish liver, however for Lake Trout (whole fish)[5] and Whitefish filet[6] the relative abundances of α- and γ-isomers were in a similar range as for Eel, Bib and Whiting in our work.

References

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