Enantiomer-specific accumulation of HBCD in fish from the Western Scheldt Estuary

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Introduction

Hexabromocyclododecane (HBCD) is used as an additive flame retardant mainly in expanded and extruded polystyrene for thermal insulation foams and to a lesser extent for backcoating of fabrics for furniture.[1] The global market consumption in 2001 was estimated around 16 700 tons of which about 57% were used in Europe.[1] HBCD has a high bioaccumulation potential and is found in increasing concentrations in environmental samples and in biota.[2,3] HBCD is produced by bromination of 1*Z*,5*E*,9*E*-cyclododeca-1,5,9-triene (*cis*,*trans*,*trans*-CDT), a butadiene trimer. The resulting technical product consists of more than 90% of a mixture of three diastereomers of 1,2,5,6,9,10-hexabromocyclododecane termed α-, β-, and γ-HBCD. The three diastereomers may be separated by reversed phase HPLC.[4] The three diastereomers are chiral and exist as pairs of mirror-image enantiomers as illustrated in Figure 1.

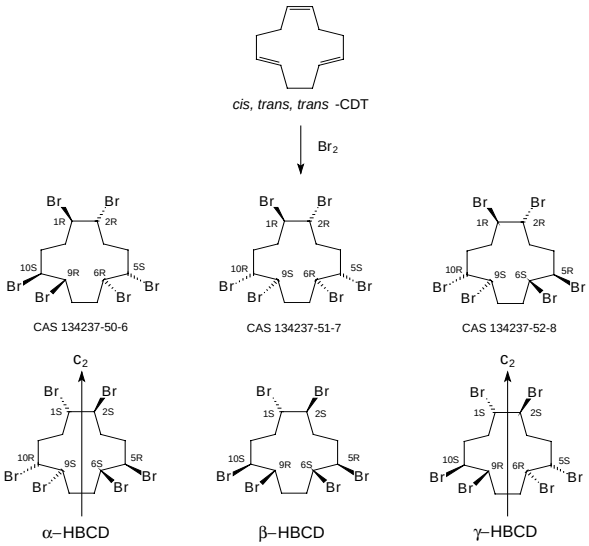

Figure 1. Stereochemical structures of the major HBCD isomers.

The enantiomers have identical physico-chemical properties and abiotic degradation rates, but may have different biological and toxicological properties and therefore different biotransformation rates. These transformations may result in nonracemic mixtures of the enantiomers. Enantiomer analysis has been used extensively to study transformation dynamics of chiral pollutants.[5] Recently the complete separation of the three pairs of enantiomers of HBCD by chiral reversed phase HPLC has been demonstrated.[6,7]

The Western Scheldt Estuary on the border between Belgium and the Netherlands has been shown to be highly contaminated with brominated flame retardants (BFRs).[8,9] Probable sources are BFR manufacturing and textile industry as well as harbour activity in Antwerp. High levels of polybrominated diphenylethers (PBDEs) have been reported in sediments and in various marine benthic and pelagic organisms.[8,9] Sediments from the Western Scheldt are also highly contaminated by HBCD.[10] In this paper, we report on the enantiomer composition of the HBCD diastereomers in various fish species from the Western Scheldt.

Materials and Methods

The sampling of different marine species from the Western Scheldt Estuary has been described previously.[8] 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 from the same location was also sampled. Sample preparation has been described elsewhere.[8]

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.

For chiral chromatography, a Macherey-Nagel NUCLEODEX beta-PM column (200 x 4.0 mm i.d.) was used. The mobile phase was 40% H_2O/30% ACN /30% CH_3OH for 0.5 min followed by a linear gradient for 7 min to 70% ACN/30% CH_3OH which was held for 14 min. The flow rate was set at 0.5 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

Chromatography of a mixture of the three diastereomers of HBCD on the chiral β-cyclodextrin column results in six peaks (Figure 2).

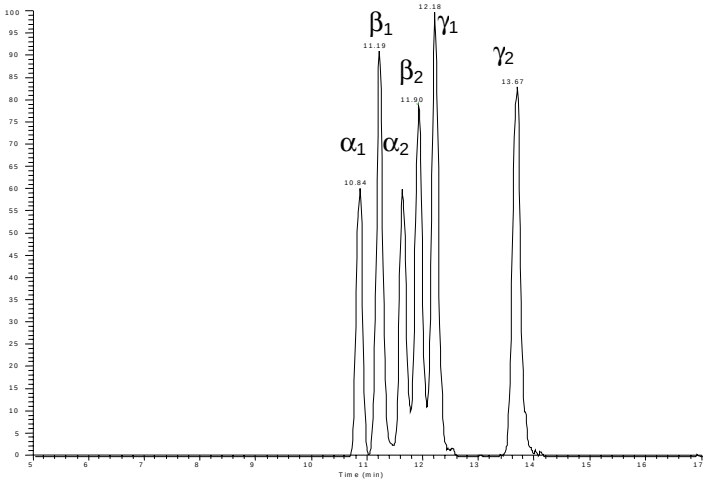

Figure 2. Chiral chromatography of a mixture of individual HBCD diastereomers.

Enantiomeric fractions (EF) were calculated from the peak areas of the enantiomeric pairs by the following formula:

$$EF = \frac{\text{Peak area enantiomer 1}}{\text{Peak area enantiomer 1} + 2}$$

EFs calculated for the standard solutions gave deviations from the value of 0.5 for pure racemic mixtures (Table 1). There are two possible reasons for these deviations. In order to allow focussing of the analytes at the column head, a mobile phase gradient was used starting with low elution strength. The mobile phase composition at the elution time of the corresponding enantiomers may differ slightly and may result in different ionisation conditions and thereby different responses. Secondly, the standards supplied may not be purely racemic when the products have been in contact with optically active materials during the purification process, e.g. cellulose filters. These alternatives are presently investigated further.

Table 1. Enantiomer fractions for the HBCD diastereomers in a mixture of standard solutions at concentrations of 200 pg/µL each.

	α-HBCD	**β-HBCD**	**γ-HBCD**
EF	0.478	0.515	0.495
RSD (n=3)	0.7	3.9	1.3

A chromatogram of the chiral separation of HBCD diastereomers in an extract of bib liver is given in Figure 3.

α_2

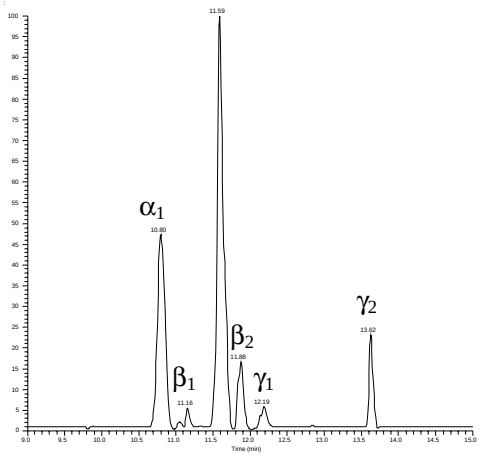

Figure 3. Chiral chromatogram of an extract of bib liver originating from the Western Scheldt Estuary.

α-HBCD is the dominating isomer, however also the β- and γ-enantiomeric pairs are clearly visible. This profile is consistent with other recent studies on the occurrence of HBCD diastereomers in aquatic organisms[11,12] and is in contrast to the profile seen in sediments where the γ-diastereomer is generally dominant[10]. The results for the fish samples from the Western Scheldt Estuary are presented in Table 2. In order to reduce the uncertainty of the results, only peak areas above 10,000 units were included in the calculation of EFs. However, indicative EFs based on areas below 10,000 are given for β- and γ-HBCD in bib liver and for α-HBCD in eel. HBCD-enantiomers could not be determined in dab and plaice fillet or liver.

Table 2. Enantiomer fractions for HBCD diastereomers in extracts from different fish species from the Western Scheldt Estuary (* denotes indicative values).

	Lipid %	**α-HBCD**	**β-HBCD***	**γ-HBCD***
Bib liver pool 1	*57*	*0.444*	*0.322*	*0.412*
Bib liver pool 2	*60*	*0.413*	*0.458*	*0.620*
Bib liver pool 3	*44*	*0.408*	*n.a.*	*0.768*
Bib mean	**54**	**0.422**	**0.390**	**0.600**
Whiting liver	**31**	**0.355**	**n.a.**	**n.a.**
Sole liver pool 1	*12*	*0.537*	*n.a.*	*n.a.*
Sole liver pool 2	*13*	*0.539*	*n.a.*	*n.a.*
Sole liver	**13**	**0.538**	**n.a.**	**n.a.**
Sole muscle	**1.2**	**0.563**	**n.a.**	**n.a.**
Eel muscle	**23**	**0.459***	**n.a.**	**n.a.**

The concentration of HBCDs was too low in the lean muscle meat of the gadoids, and therefore only results for liver are presented. For both species, the first α-enantiomer is considerably lower than the second one indicating a strong enantioselective bioaccumulation. For sole, peak areas of α-HBCD enantiomers peak areas were high enough for EF calculation in both liver and muscle. This observation is consistent with what was previously reported for sole concerning PBDEs.[8] In contrast with the other fish species, sole did not preferentially accumulate PBDEs in the liver, resulting in relatively high levels in the muscle tissue. Although levels of HBCD are comparable in all fish levers, HBCD could not be determined in any muscle sample, except in sole muscle. This indicates a similar HBCD tissue distribution as for PBDEs when compared to the other fishes.

In sole, enantiomeric accumulation seems to be the same for liver and muscle with the first α-enantiomer being more abundant than the second one. For eel muscle, no obvious enantioselective accumulation was observed. Both β- and γ-isomers have very low abundance in all samples and the EFs calculated for bib liver are subjected to relatively large uncertainty. Nevertheless, it seems consistent, that EFs were < 0.5 for β-HBCD and > 0.5 for γ-HBCD.

Conclusion

We reported here for the first time on the enantiomer-specific determination of HBCD diastereomers in marine biota from a polluted estuary. The chiral LC-MS-MS method allowed the determination of enantiomeric fractions for α-HBCD, the most abundant diastereomer. The results show that enantioselective accumulation of HBCD is species dependent.

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