Enantiomer-Specific Accumulation of Hexabromocyclododecane in a Lake Ontario Food Web

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Introduction

Hexabromocyclododecane (HBCD) is the most widely used of the cycloaliphatic brominated flame retardants (BFRs), and is an additive flame retardant used in polystyrene foams and upholstery textiles. Technical 1,2,5,6,9,10-HBCD is produced by bromination of cis, trans, trans cyclododecatriene (CDT); the resulting mixture contains three predominant diastereomers (α-, β-, and γ-HBCD).[1] These diastereoisomers exhibit different physico/chemical properties (e.g., water solubility) that can influence rates of biological uptake and metabolism. Recent reports provide evidence of bio-isomerization of the diastereoisomers in fish with a preferential formation of the α-isomer.[2,3,4] The individual α-, β-, and γ-HBCD diastereoisomers are represented by a corresponding pair of enantiomers. In general, industrially synthesized chiral pollutants are produced as racemates, and correspondingly released into the environment as racemates.[5] The integrity of the racemic mixture is maintained when subjected to achiral interactions such as hydrolysis, photolysis, leaching, volatilization and atmospheric deposition. However, alterations in enantiomeric composition might occur as a result of biological processes such as metabolization.[5]

Given the current interest in HBCD and its role as an environmental contaminant, an understanding of the environmental and biological fate of HBCD enantiomers is required. An enantiomer-specific method using chiral-phase HPLC coupled with tandem mass spectrometry has been reported and applied to the investigation of HBCD enantiomers in fish from the Western Scheldt Estuary in Belgium.[5] The authors reported significant enantioselectivity for both α- and γ-HBCD in some biota samples. We have used similar methodology in the current investigation of enantiomer-specific accumulation of HBCD in a Lake Ontario food web using LC-MS-MS with electrospray ionization (ESI) in negative ion mode. We also previously reported that a number of factors influence accurate quantitation of HBCD diastereoisomers in complex environmental mixtures, including matrix effects and instrument response.[8] This current work also serves as an investigation of factors influencing quantitation of HBCD enantiomers, and therefore on the corresponding enantiomeric fraction (EF) calculations.

Materials and Methods

Lake Ontario pelagic food web samples (lake trout, alewife, rainbow smelt, slimy sculpin, mysids, and amphipods) were previously analyzed for HBCD diastereomers[3]; these same extracts were used for the enantiomer-specific study.

Fish (8-15 g wet weight) samples were extracted by accelerated solvent extraction, lipids were removed by gel permeation chromatography and the extracts were subjected to Florisil chromatography. The LC method for the separation of HBCD enantiomers was based on that of Janak et al.[5] Chromatographic separation was preformed on an Agilent 1100 Series LC system (Mississauga, ON) using a chiral LC column (4.0 x 200 mm, 5 µm) containing permethylated [β]-cyclodextrine on silica -NUCLEODEX beta-PM- (Macherey-Nagel, Germany). Samples (2 µL injected) were analyzed on a MDS/Sciex 4000 QTrap hybrid triple quadrupole /linear ion trap mass spectrometer (Concord, ON) in electrospray ionization negative ion mode using multiple reaction monitoring (MRM) for the $[M-H]^-$ (m/z 640.6) $\rightarrow Br^-$ (m/z 78.9 and 80.9) transition. The mobile phase consisted of water, methanol and acetonitrile at a constant flow rate of 500 µL per minute. The initial solvent conditions were 42% water/30%

methanol/28% acetonitrile. The solvent composition was changed linearly over 14 minutes to a final solvent composition of 30% methanol/70% acetonitrile. The final conditions were held for 6 minutes before a linear ramp to the initial solvent conditions and the column was equilibrated for 30 minutes between analyses. Using a binary pump this gradient was achieved with solvent mixtures (A) 30% methanol/70 % water and (B) 30% methanol/70% acetonitrile.

Results and Discussion

The chiral signature of HBCD was expressed as the enantiomeric fraction (EF), defined as $EF = A_+/(A_+ + A_-)$ where A_+ and A_- are the first and second eluting enantiomer, respectively. Previous work identified the (-) α, (-) β and (+) γ HBCD as the first eluting peak of each corresponding enantiomeric pair.[6] A racemic compound in theory will have an EF = 0.5; any significant deviation from 0.5 indicates a shift in enantiomeric composition.

The analysis of pure HBCD standard containing the three diastereoisomers by ESI LC-MS-MS (Figure 1) resulted in EFs slightly lower than 0.5 for α- and β-isomers but higher than 0.5 for the γ-isomer. This effect was observed for a range of concentrations (20-200 pg on column), and was most pronounced for γ-HBCD at higher concentrations (Table 1). However, variability of the EFs was greater at lower concentrations. Analysis of the ^{13}C- and d_{18}- labeled HBCD diastereoisomers gave similar results. Given that the HBCD standard mixture is racemic, variations in EFs from 0.5 are presumably due to differences in instrument response, with the potential additional influence of matrix effects in environmental samples. As a result, this apparent deviation from the theoretical EF of 0.50 should be considered when quantifying HBCD enantiomers in environmental samples.

Table 1. Enantiomeric fractions (EFs) for HBCD diastereoisomers in pure standards.

	α-HBCD	β-HBCD	γ-HBCD
	EF (n=7)	EF (n=7)	EF (n=7)
Individual HBCD isomers (50 pg/uL)	0.48 ± 0.01[a]	0.47 ± 0.01	0.64 ± 0.01
HBCD standard mixture (100 pg/uL)	0.48 ± 0.01	0.47 ± 0.02	0.62 ± 0.02
HBCD standard mixture (25 pg/uL)	0.50 ± 0.03	0.51 ± 0.02	0.56 ± 0.01
HBCD standard mixture (10 pg/uL)	0.49 ± 0.04	0.49 ± 0.04	0.55 ± 0.03

[a]arithmetic mean +/ standard deviation

Table 2. Corrected enantiomeric fractions (EFs) for HBCD in Lake Ontario lake trout

Sample	EF	EF	EF	EF
	α-HBCD uncorrected	α-HBCD corrected	γ-HBCD uncorrected	γ-HBCD corrected
Lake Trout #1	0.43	0.51	0.68	0.52
Lake Trout #2	0.39	0.47	0.62	0.54
Lake Trout #3	0.43	0.50	0.65	0.54
Lake Trout #4	0.40	0.51	0.53	0.57

Quantitation of HBCD diastereoisomers in the Lake Ontario food web samples were previously reported.[2] The α-isomer was consistently higher than the γ-isomer, while the β-isomer was below method detection in all samples (Figure 2). Corresponding EFs for selected Lake Ontario food web samples are shown in Table 2. The EFs shown in Table 2 are "corrected" values, i.e., individual HBCD enantiomers were quantified based on corresponding d_{18}-labelled analogues added prior to injection as instrument standards. Although the "uncorrected" profile shown in Figure 2 would suggest enantioselectivity for the Lake Ontario lake trout samples, i.e., a predominance of the (-) α-enantiomer over the (+) α-enantiomer, the corrected values in Table 2 show the true EFs are much closer to being racemic. This clearly highlights the need for using internal standards to benchmark the EF values. However, corrected EFs for the γ-HBCD enantiomers indicate the potential for enantioselectivity of these isomers, as the EF values were all substantially greater than 0.5.

The issue of enantioselectivity at multiple levels within a food chain will be investigated further through a more exhaustive statistical study of all of our Lake Ontario biota samples. However, this preliminary work has clearly identified the potential for misinterpretation of HBCD EF data resulting from a lack of consideration of the potential impacts of instrument response and matrix effects.

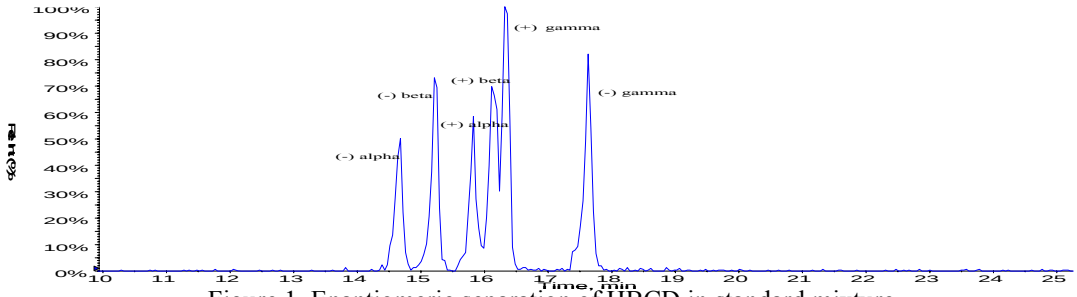

Figure 1. Enantiomeric separation of HBCD in standard mixture

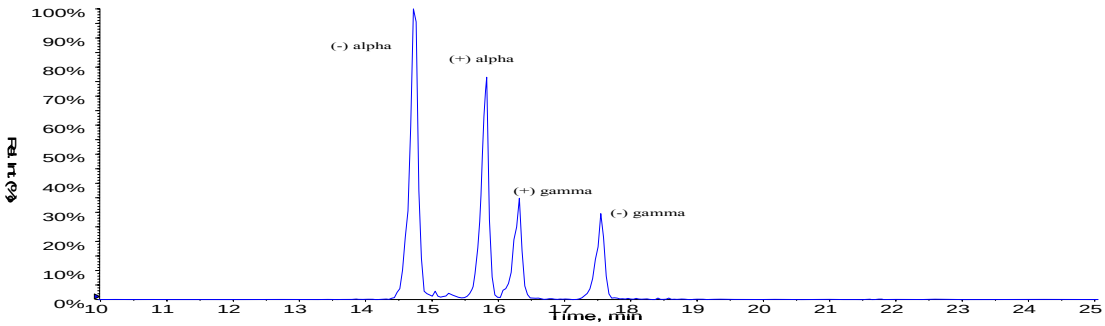

Figure 2. Enantiomeric separation of HBCD in Lake Trout from Lake Ontario

References

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