Organohalogen Compounds, Volume 61, Pages 155-158 (2003)

Detection of α-isomer dominated HBCD (hexabromocyclododecane) in Swiss fish at levels comparable to PBDEs (polybrominated diphenyl ethers)

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Introduction

HBCDs (hexabromocyclododecanes) are used as flame retardants for plastics such as rigid insulation panels, electric housings, and for textile back coatings. The world-wide market demand for HBCDs was estimated to 16700 tons in 2001[1]. Commercial HBCD is a mixture of isomers of 1,2,5,6,9,10-hexabromocyclododecane. Commonly three isomers, denoted as α-, β-, and γ-HBCD[2,3], are reported. HBCDs have not shown any acute toxicity below their water solubility for fish, daphnia and algae[2]. However, physiological effects at the cell level were reported[4,5]. Data on chronic effects are rather scarce and the implications of the high bioaccumulation factor (log BCF ~ 4[2,6]) of HBCDs are not known, yet. The European Union is currently undertaking a risk assessment for HBCDs under its existing substances regulation 793/93/EEC. Very limited data are available on environmental concentrations of HBCDs. Studies from Sweden reported lipid based concentrations of HBCDs between < 50 and 8000 ng/g lipid weight (lw) in fish and guillemot eggs[7,8]. No information on the isomer distribution was given.

This paper reports α-, β-, and γ-HBCD concentrations in Whitefish samples from six Swiss lakes. The advantages of different analytical techniques (LC/MS versus GC/MS) as well as the implications of the results are discussed. To our knowledge, this is the first report on individual concentrations of α-, β-, and γ-HBCD in environmental samples.

Materials and Methods

HBCD reference materials (α-, β-, and γ-HBCD) were obtained from Cambridge Isotope Laboratories. Whitefish (*Coregonus sp.*) samples from six Swiss lakes (8 - 582 km^2 surface area) were pooled (filets of 10 individuals) and stored at -20°C until analysis[9]. Aliquots (10 g) of the homogenized samples were mixed with 100 g Na_2SO_4 and extracted with a 1:1 mixture of hexane/acetone (soxhlet extraction, 14 h). The concentrated extract was treated with sulfuric acid, partitioned with 0.5 M K_2CO_3 to separate weak acids for later analysis, and purified on a silica gel column (6 g silica gel 60, deactivated with 10% water; rinsing steps: 30 ml hexane and 20 ml hexane/dichloromethane 92:8; elution step: 30 ml hexane/dichloromethane 1:1). Prior to analysis, solvents were changed to methanol/water (80:20) for LC- and to toluene for GC-analysis. HBCD isomers were analyzed on a C_{18}-reverse phase LC-column (125 mm x 4 mm, Macherey-Nagel) on a TSP 4000/1000 LC-system (Thermo Separation Products). The mobile phase was methanol/water (80:20) at a flow rate of 1 ml/min. Negative ion APCI-MS spectra were acquired in selected ion monitoring mode on a TSQ 7000 triple stage quadrupole mass spectrometer (Thermo Finnigan). GC analysis was carried out using a DB-1 equivalent column (10 m × 0.28 mm, film 0.1 μm). Samples were injected on-column (1 μl). Positive ion EI-MS spectra were acquired on a MAT 95 high resolution mass spectrometer (Thermo Finnigan MAT) in single ion monitoring mode at an ionization energy of 60 eV and a mass resolution of 8000.

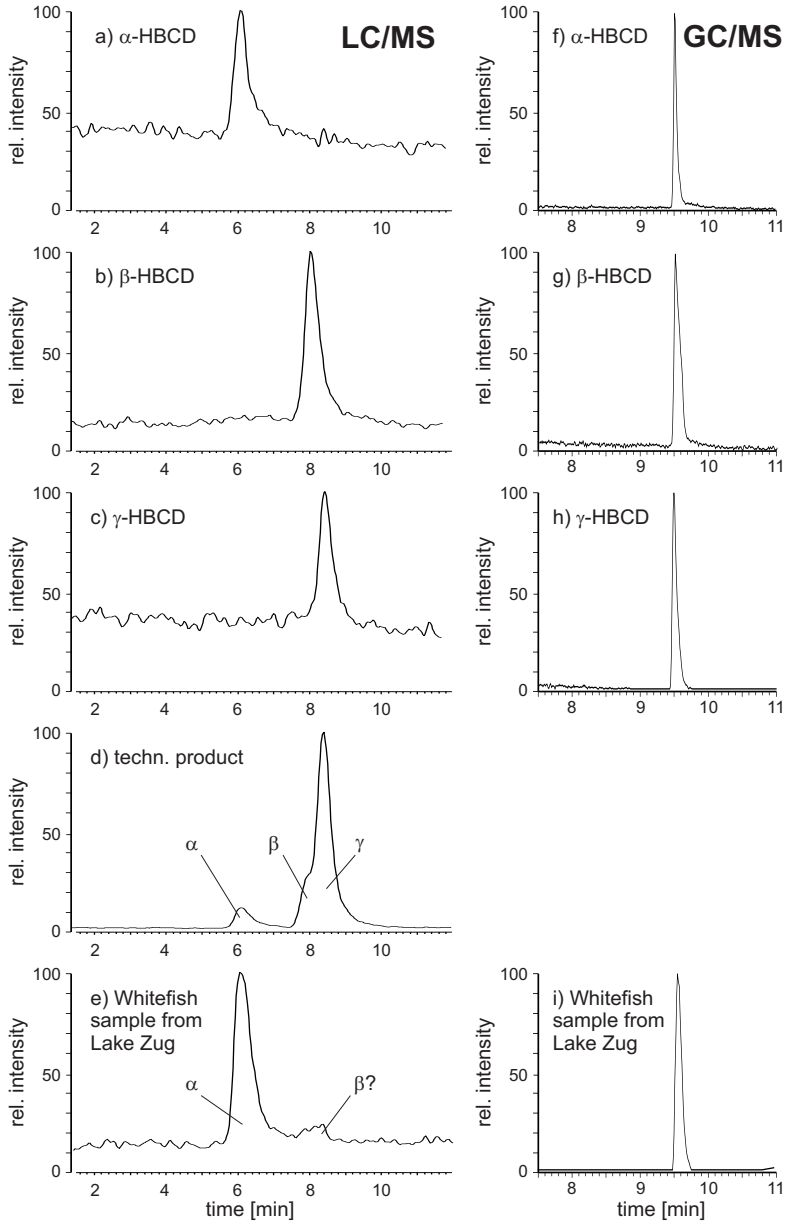

Figure 1. LC/MS and GC/MS chromatograms of standard solutions and a Whitefish sample. HBCD on column: 2.5 ng in Fig. a, b, and c, and 0.4 ng in Fig. f, g, and h.

Results and Discussion

Analytical methods. The recovery for the analytical procedure was 62 ± 6% (n=11). The resulting GC/MS chromatograms were free from major interferences (see Fig. 1i). LC/MS chromatograms occasionally exhibited some matrix peaks. The instrumental quantification limit (signal-to-noise ratio of 10:1) of the GC/MS method (0.03 - 0.05 ng on column) was about 20-times

Table 1. *Comparison of chromatographic and instrumental parameters (LC/MS vs. GC/MS).*

	monitored masses [m/z]	retention time [min]	peak width at half height [min]	IQL[a] [ng on column]	sensitivity relative to γ-isomer [ ]
LC/MS	$[M - H]^-$				
α-HBCD	638.6; 640,6; 642,6	6.1	0.40	1.0	1.7
β-HBCD	"	7.9	0.42	0.5	2.7
γ-HBCD	"	8.4	0.40	1.1	1
GC/MS	$[M - Br]^+$				
α-HBCD	560.7289; 562.7269	9.52	0.05	0.03	1.09
β-HBCD	"	9.53	0.11	0.05	1.05
γ-HBCD	"	9.51	0.06	0.03	1

[a] *IQL = instrumental quantification limit (S/N = 10); signal and noise (standard deviation of base line) determined in chromatograms of standard solutions (2.5 ng on column for LC/MS and 0.4 ng on column for GC/MS).*

lower than the instrumental quantification limit the of the LC/MS method (0.5 - 1.1 ng on column, see Table 1). On the other hand, only the LC-method was able to (partially) separate the three HBCD-isomers, as shown in Figure 1. Nevertheless, it was possible to quantitatively measure the sum of these three coeluting isomers (ΣHCBDs) by GC/MS, because the three isomers exhibited a similar sensitivity in the MS (Table 1). Quantitative LC/MS and GC/MS results showed a very good agreement (Table 2). No indications of thermal degradation were observed in GC/MS chromatograms. However, the broad signal of β-HBCD in the GC/MS chromatogram (see Fig. 1g) may indicate a partial conversion of β-HBCD into other isomers, as reported by Peled et al.[10]

HBCD concentrations in Whitefish. Lipid based ΣHBCDs concentrations in Whitefish filet from six Swiss lakes varied between 25 and 210 ng/g lw. Sellström et al.[7] reported concentrations of HBCDs in muscle tissue from pike (*Esox lucius*) below 100 ng/g lw upstream of textile industries but up to 8000 ng/g lw downstream of textile industries, which were a likely point source for HBCDs. In comparison with these big differences upstream and downstream of suspected point sources, the variations of ΣHBCDs concentrations in Whitefish from the six Swiss lakes were low. Therefore, we assume that there were no major local sources present in the catchment areas and that the HBCDs detected in our fish samples originated from multiple diffuse sources. The ΣHBCDs concentrations were in the same range as the PBDE levels[9] in the investigated Whitefish samples (see Fig. 2). Comparison of these PBDE levels with PBDE concentrations in fish samples from other countries indicated, that there were also no major local sources for PBDEs in the catchment areas of the investigated Swiss lakes, either.

Table 2. *Concentrations of individual HBCD isomers in Whitefish from Swiss lakes.*

sampling site	lipids [%]	GC: ΣHBCD [ng/g lw][a]	LC: α-HBCD [ng/g lw][b]	LC: β-HBCD [ng/g lw][c]	LC: γ-HBCD [ng/g lw]	LC: α-HBCD [%]
Lake Zug	2.6	210	210	<9	<18	**> 88**
Lake Zürich	3.5	130	n.a.	n.a.	n.a.	n.a.
Lake Greifen	3.8	85	100	<6	<12	**> 85**
Lake Sempach	1.5	64	66	<15	<33	**> 58**
Lake Neuenburg	7.2	48	54	<3	<6	**> 86**
Lake Geneva	5.3	25	n.q.	n.q.	n.q.	n.q.

n.a. = not available, n.q. = no quantification possible due to exceptional matrix noise; [a] *n=2, except for Lake Sempach (n=1), relative difference between duplicates always < 10%;* [b] *n=1;* [c] *Different lipid based quantification limits resulted from the different lipid content of the individual samples.*

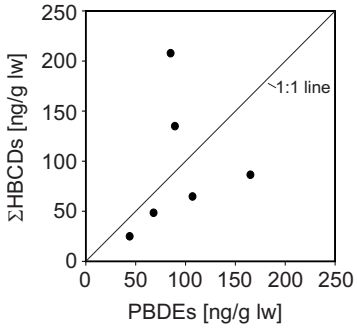

Figure 2. ΣHBCDs concentration versus the sum of PBDEs (sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183[9]) in Whitefish samples from six Swiss lakes.

HBCD isomer pattern in Whitefish samples. In all four fish samples analyzed for HBCDs by LC/MS, **the α-isomer clearly dominated the isomer pattern** (Table 2). Contrary, commercial HBCD is typically dominated by the γ-isomer (see Fig. 1d and ref.[2]). Thus, at least one of the processes involved into the transfer of HBCDs from manufacturing, application, and/or disposal sites to the fish filet must be isomer specific. Partitioning, chemical degradation, and biological transformation processes are potentially isomer specific. At this point, we can only speculate on the nature of the involved isomer specific process(es). Next to biotic processes being responsible for the relative enrichment of the α-HBCD isomer, it might be possible that the relative enrichment is at least partly based on physical properties, such as water solubility. The α-HBCD isomer is the least hydrophobic (given the elution order on reverse-phase LC columns) and might be more prone to leach from flame retarded materials. A similar relative enrichment of α-HBCD was observed in rats after oral administration of technical HBCD[2].

Possible implications for future studies and regulations. Our results show that the different HBCD isomers do have a different environmental fate and hence may have a different environmental impact. Therefore, we suggest that future environmental and toxicological studies on HBCDs should, whenever possible, be carried out individually for the different isomers. Results of such research could lead to conclusions that certain technical mixtures, rich or low in a specific isomer, do have a lower environmental impact than others.

Acknowledgements

We acknowledge the Swiss National Science Foundation for financial support (Project FLARE, 4050-066536, National Research Program 50).

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