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Distribution and Fate of HBCD and TBBPA Brominated Flame Retardants in North Sea Estuaries and Aquatic Food Webs

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Tetrabromobisphenol A (TBBPA) and hexabromocyclododecane diastereoisomers (α-, β-, and γ-HBCD) were investigated in effluents from sewage treatment works, landfill leachates, sediments, and food web organisms of the North Sea basin. Residues were quantified by liquid chromatography–mass spectrometry. Both flame retardants were enriched in sewage sludges, where a maximum total (Σ) HBCD concentration of 9.1 mg/kg (dry weight; d.w.) was found; TBBPA was at levels of 10^2 μg/kg. Landfill leachates from The Netherlands showed up to 36 mg (ΣHBCD)/kg (d.w.). γ-HBCD dominated isomeric profiles in sediments, and concentrations were elevated near to a site of HBCD manufacture. α-HBCD was the primary congener detected in marine mammals; however, very few samples exhibited TBBPA. ΣHBCD ranged from 2.1 to 6.8 mg/kg (lipid weight; l.w.) in liver and blubber of harbor porpoises (*Phocoena phocoena*) and seals (*Phoca vitulina*). TBBPA levels in cormorant (*Phalacrocorax carbo*) livers were up to 1 order of magnitude lower compared to ΣHBCD. HBCD in eels (*Anguilla anguilla*) from the Scheldt basin (Belgium) reflected the spatial distribution of concentrations in local sediments. This study shows evidence of HBCD bioaccumulation at the trophic level and biomagnification in the ascending aquatic food chain, and these findings justify risk assessment studies at the ecosystem level.

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Introduction

Brominated flame retardants (BFRs) are comprised of several different, high production volume chemicals that are used to inhibit or impede flammability in combustible products (*1*). Hexabromocyclododecane (HBCD, Figure 1) and tetrabromobisphenol A (TBBPA, Figure 2) are two BFRs currently in use. HBCD's main application is to flame retard extruded and expanded polystyrene that is used as thermal insulation in buildings. A minor application for HBCD is in upholstery textiles. The HBCD technical product is composed of three diasteroisomers (α, β, and γ), with γ-HBCD contributing to approximately 80% of technical formulation (*2*). TBBPA is the primary flame retardant used in electronic circuit boards and is covalently bound to the resin (*2*). Decabromodiphenyl ether (deca-BDE) and TBBPA account for approximately 50% of the world's usage of BFRs. TBBPA is the most widely used BFR and in 1999, 13 800 tonnes (t) of TBBPA and 8900 t of HBCD were consumed in the European Union (EU) (*3*). The usage of BFRs appears to differ among the countries or regions of the world (*4*).

The widespread, environmental distribution of lower BDEs is well-known, and publications have focused on that class of BFRs (see ref *5* for review). Few studies report the presence of HBCD and TBBPA in the environment. For example, HBCD has been detected in indoor dust (*6*), and TBBPA has been found in the interior of television sets (*7*). TBBPA has also been reported in sludge collected from sewage treatment works (STWs) and at concentrations up to 50 μg/kg (dry weight; d.w.) (*8*, *9*). The levels of HBCD, TBBPA, and BDEs in river sediments sampled in the vicinity of Swedish textile plants and a plastics industry applying these BFRs were higher compared to concentrations upstream of discharge points (*10*, *11*). TBBPA has also been reported in sediment (*12*). Tissues from mussels acquired downstream of a BFR production facility in The Netherlands showed HBCD concentrations up to 51 μg/kg (lipid weight; l.w.) (*13*). A mean concentration of 124 μg(HBCD)/kg (l.w.) was found in Guillemot eggs from the Baltic Proper (*14*), and temporal trends studies of residue levels indicated a significant increase from 1969 to 1997 (*15*). After 1997, an EU risk assessment evaluated this study and concluded that the trend was leveling off. HBCD has also been reported in arctic air and biota (*16*). Although the evidence for the environmental presence of HBCD and TBBPA is small, their detection in a wide range of matrices alerts us to a potential environmental concern.

Previously published HBCD concentration data have been derived by gas chromatography (GC) with either negative ion chemical ionization mass spectrometry or GC with electron capture detection (*17*). Analysis by GC has limitations as the three diastereomers cannot be resolved chromatographically (*2*), and they are thermally labile (*18*) at temperatures (>160 °C) that are commonly applied during GC separation. Thus, HBCD values have been reported as total (Σ) HBCD, although visible chromatographic peak broadening caused by thermal instability has resulted in less accurate data. Through the application of liquid chromatography mass spectrometry (LC-MS), it is now possible to separate α-, β-, and γ-HBCD diastereomers (*19*–*21*). To the best of our knowledge, this is the first study to deploy LC-MS for the simultaneous detection and quantification of the individual HBCD diastereomers, and TBBPA in an extensive range

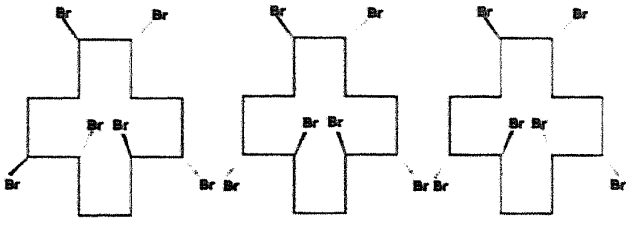

FIGURE 1. Chemical structures of the α-, β-, and γ-diastereomers (after 18) of 1,2,5,6,9,10-hexabromocyclododecane (HBCD) and their *R*–*S* configurations.

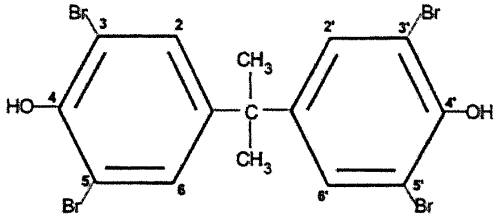

FIGURE 2. Chemical structure of tetrabromobisphenol A (TBBPA, 2,2-bis(4-hydroxy-3,5-dibromophenyl)propane).

of biotic and abiotic compartments from the aqueous environment. The identification of possible sources of these BFRs is also described here.

The purpose of this research was to examine the occurrence of HBCD diastereomers and TBBPA in effluents from STWs and landfill leachates and to determine concentrations and diastereomeric profiles in sediments from rivers and estuaries surrounding the North Sea. A further purpose was to investigate their bioaccumulation in organisms representing the different trophic levels of the North Sea food web. Three laboratories undertook comparisons of the LC-MS technique and concluded that harmonized methods were sufficiently robust to perform this study (*22*).

Experimental Section

A sample location map of sediment and biotic taken from the rivers and estuaries of the North and Irish Seas and Scheldt basin is presented in Figure 3.

Sampling of Eels and Sediments in the Scheldt Basin (Belgium). Yellow eels (*Anguilla anguilla*) were taken in 2000 and from 16 locations in the Scheldt Basin and from three reference sites (a: Warmebeek, Achel-kluis; b: Grote Beverdijk, Lo-Reninge; c: R. IJzer, Nieuwpoort). Either electro- or fyke fishing techniques were used to collect the eels. At each sampling location, tissue from 10 individual eels (30 to 50 cm length) were pooled prior to homogenization and analysis. Samples of sediment were collected in August 2001 from the same 19 locations with an additional sample taken at Vrasenedoc in Beveren, Belgium. Ten sediment subsamples were acquired at each site and by a van Veen grab, and these were pooled and thoroughly homogenized prior to extraction. Sample location details are given as Supporting Information in Table SI-1.

Sediment and Food Web Samples from the Western Scheldt, The Netherlands. In 2000, sediment samples from 19 locations in the Western Scheldt were acquired by a van Veen grab (see Supporting Information Table SI-2). Sediment samples from nine Dutch rivers (see Table SI-3) were also taken during the same sampling period; yellow eels from the same rivers had been collected in 1999 and by the methods described above. At Terneuzen, eggs (n=10) from the common tern (*Sterna hirundo*), one sample of mysid shrimp (*Crangon crangon*), and one of sand goby (*Pomatoschistus minutus*) were taken in 2001. Tern eggs were analyzed on an individual basis.

Collection of Invertebrates, Fish, and Marine Mammals from the North Sea. Details of the sampling and the preparation of samples of common whelk (*Buccinium undatum*), sea star (*Asterias rubens*), hermit crab (*Pagurus bernhardus*), the gadoid fish species whiting (*Merlangius merlangus*) and cod (*Gadus morhua*), and the marine mammal species harbor seal (*Phoca vitulina*) and harbor porpoise (*Phocoena phocoena*) are described elsewhere (*23*). Invertebrates and fish were taken during a research vessel (RV *Pelagia*) cruise (August–September 1999), and the following tissues were used: soft tissue (whelk, n= 3), part of the intestinal system or *pyrloric caeca* (sea star, n=3), abdomen (Hermit crab, n=9), muscle (whiting, n=3). Samples of marine mammals were taken from stranded or by-caught individuals, and the following tissues were analyzed on an individual basis: liver (harbor porpoise, n=1), blubber (h.

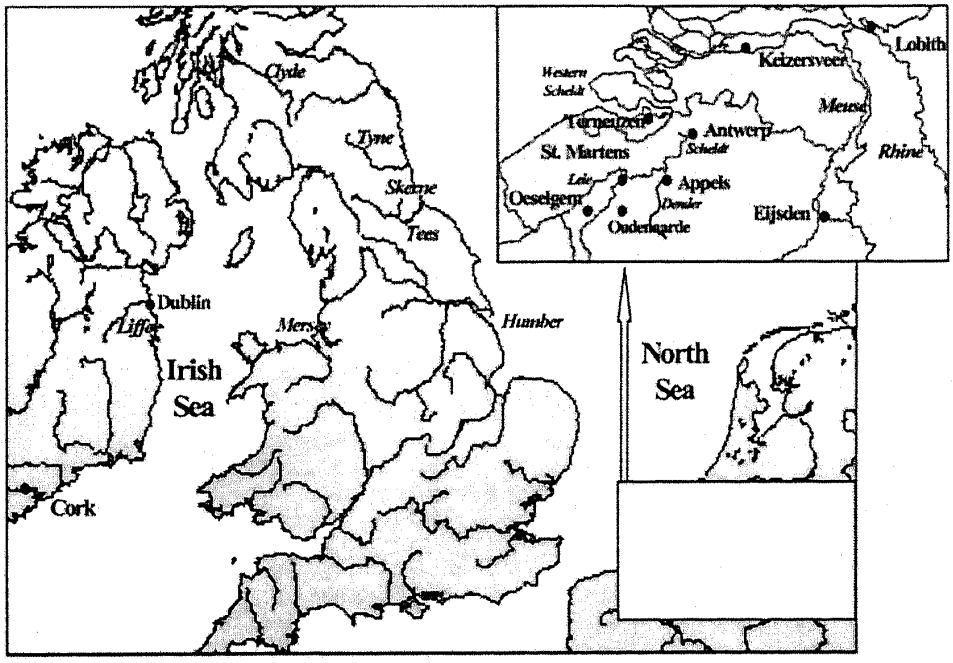

FIGURE 3. Location of sampling sites. The inset map details the Dutch and Belgium rivers sampled for sediments and yellow eels.

porpoise, n=4), liver (h. seal, n=3), and blubber (h. seal, n=2).

Sampling of River Sediments and Marine Biota from the United Kingdom. Sediment samples were obtained in 2000, by a hand-held Day grab and from the rivers Skerne, Tees, Tyne, and Humber (northeast England), Clyde (Scotland), and the Mersey (northwest England, and collected in 2002). The upper 10 to 15 cm of each sediment sample was acquired. Details of the sampling locations are given as Supporting Information in Table SI-4. In June 2001, three whiting (muscle) and a single sea star (whole tissue) were taken within the mouth of the Tees estuary and with a 5 m beam trawl during the RV *Cirolana* cruise. All porpoise blubber samples were acquired in 1998 and from stranded or by-caught animals. Cormorant (*Phalacrocorax carbo*) livers were taken in 1999 and 2000 and from individuals acquired under the U.K.'s Home Office License. A summary of the sample details including the age and sex of porpoises and cormorants are presented in Supporting Information Table SI-5.

Sampling of Sewage Sludge and Landfill Leachates. Influents, effluents, and sewage sludges were taken in 2002, from STWs in The Netherlands, United Kingdom, and the Republic of Ireland. A total of seven STWs were sampled in The Netherlands, with four facilities serving conurbations with populations in the range of 100 000 to 400 000; the remaining were obtained from STWs receiving sewage from smaller communities with populations of approximately 10 000. Five STWs from southeast England were sampled serving populations in the range of 4800 to 143 000. All STWs sampled in this study received domestic wastewater only. Influents and final effluents (2.5 to 4 L) were filtered using 0.45 μm polyvinyl disk filters (BDH, Dorset, U.K.) to obtain the dissolved and particulate phases. Isolated suspended particulates and sewage sludges were dried at 50 °C prior to extraction. Only sewage sludge was taken from three STWs in Ireland; Portlaoise, Clonmel, and Cork. Leachates from landfill sites in receipt of domestic or municipal wastes only were also obtained in 2002, and from nine locations in The Netherlands, three from southeast England, and three from Ireland. No industrial or chemical wastes had been received at any of the study sites. Leachates were treated before their discharge to adjacent watercourses by primary settlement, and samples were taken prior to their treatment. As with influents and effluents from STWs, samples (1 to 2.5 L) were filtered to acquire the dissolved and particulate phases, and the latter was dried as above.

Sample Extraction and Cleanup. All solvents used in the extraction and analysis procedures were of HPLC-grade quality. Reagent grade ammonium acetate and anhydrous sodium sulfate were purchased from Sigma-Aldrich Co. Ltd. (Dorset, England). Stock solutions (50 μg/mL) of α-, β-, and γ-hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA), and $^{13}C_{12}$ labeled TBBPA were obtained from Promochem (Hertfordshire, England).

Each of the three laboratories participating in this study used different sample extraction and cleanup procedures, and these have been reported elsewhere (*24*). In brief, liquid–solid extractions by Soxhlet or homogenization by Ultra Turrax using binary solvent mixtures (1:1 or 1:3 (v/v) acetone: n-hexane mixture) were performed on sediments and biota.

Following the addition of the $^{13}C_{12}$ labeled TBBPA surrogate standard to an aliquot of the crude extract, extracts were shaken with concentrated sulfuric acid to degrade coextracted lipid material. The extract was then transferred to a gel permeation chromatography system with two, cross-linked divinylbenzene gel columns in series (*24*). Analytes were eluted with dichloromethane and reconstituted in isooctane. This extract was then fractionated using silica gel column chromatography (Merck Kieselgel 60, 63–200 μm; no. 7754; BDH, Dorset, England). The first fraction (11 mL of isooctane) contained BDEs, and the second fraction (25 mL of 15% v/v diethyl ether in isooctane) contained the HBCD diastereomers and TBBPA. The latter fraction was reduced to just dryness, reconstituted in methanol, and stored at −20 °C prior to analysis.

Analysis. Six working calibration standards (0.003 to 0.15 μg/mL) were prepared in methanol by combining and then diluting the individual HBCD and TBBPA stock solutions. Analysis involved liquid chromatography–mass spectrometry (LC-MS), and a Platform II single quadrupole MS (Waters-Micromass, Manchester, England) was coupled to a HP1050 LC (Agilent Technologies U.K. Ltd., Cheshire, England) by an electrospray ionization source. A 2.0 × 150 mm (5 μm) Luna C18 column *plus* a guard column (Phenomenex, Cheshire, England) were used to separate the analytes. A flow rate of 250 μL/min and a gradient program consisting of two mobile phases, (A) water and (B) acetonitrile, each with 10 mM ammonium acetate were applied. An initial mobile phase composition of 80% (A) was decreased to 13% over 25 min, and this was then maintained for a further 10 min and then ramped to 80% (A) at 36 min. The column was equilibrated for a further 14 min and was maintained at 40 °C. Optimized cone voltages of −40 and −25 V were established for TBBPA (including $^{13}C_{12}$ TBBPA) and for α-, β-, and γ-HBCD, respectively. Selected ion monitoring of the deprotonated analyte ions $[M-H]^-$ was then performed in retention time, scheduled events. The selected ions were m/z 640.7 for the HBCDs, 540.9 for TBBPA, and 554.8 for $^{13}C_{12}$ TBBPA. The capillary voltage and source temperature were held at −3.5 kV and 150 °C, respectively. Concentrations of HBCDs and TBBPA in sample extracts were determined by external and internal quantification, respectively. Total (Σ) HBCD values were obtained by the summation of the three diasteroisomers.

The lowest limits of quantitation for the HBCDs and TBBPA were 0.15 and 0.05 ng on column, respectively. Using an intake mass of 1 g of biota or sediment and a 15 μL injection volume, limits of detection were 1.2 and 0.5 μg/kg for HBCDs and TBBPA. A laboratory reference material (Brown trout) incurred with HBCDs was extracted and analyzed on six separate occasions, and a coefficient of variation for ΣHBCD of ±4.5% was determined. The accuracy of the extraction and cleanup methods was measured by fortifying n-hexane with individual HBCDs to produce a mass-on-column of 2.25 ng for each diastereomer. Mean recoveries (and their ranges; n=6) were as follows: for α-HBCD 84% (68–104%); for β- 66% (47–87%); and for γ- 82% (69–96%).

Results and Discussion

Ranges and mean (± one standard deviation) values of ΣHBCD and TBBPA concentrations for the different sample matrices are presented in Table 1. Concentration data for the HBCD diastereomers as well as TBBPA are given for selected biotic and abiotic samples, and these are shown in Table 2.

Occurrence of HBCD and TBBPA in Effluents from Sewage Treatment Works. Possible sources of these BFRs to the aquatic environment include final effluent discharges from STWs. For U.K. samples, both ΣHBCD and TBBPA were present in STW influents, and, for the former compound, levels were measured in the particulate fraction (<0.4 to 29 μg/kg d.w; Table 1) as well as in the dissolved phase (up to 24 ng/L; Table 2). As a product of its hydrophilic nature, TBBPA was detected primarily in the dissolved phase of U.K. STW influents and a range of 2.6 to 85 ng/L were found. As expected, HBCDs were enriched in settled sludges, demonstrating their association with suspended particulate material and their removal during wastewater treatment.

TABLE 1. Range and Mean Concentrations Including One Standard Deviation of Total (Σ)HBCD and TBBPA in Aquatic Biota (μg/kg Lipid Weight) and Sewage, Landfill Leachates, and Sediments (μg/kg Dry Weight)

			ΣHBCD		TBBPA	
sample type	location	n	range	mean (s.d.)	range	mean (s.d.)
		Aquatic Biota				
common whelk (whole)	North Sea	3	29–47	35 (10)	5.0–96	45 (46)
sea star (digestive system)	Western Scheldt	3	<30–84	44 (42)	<1–2	4 (5)
hermit crab (abdomen)	North Sea	9	<30		<1–35	11 (15)
whiting (muscle)	North Sea	3	<73		<97–245	136 (125)
cod (liver)	North Sea	2	<0.7–50		<0.3–1.8	
hake (liver)	Atlantic – S. Ireland	1	<0.6		<0.2	
eel	Scheldt basin, Belgium	19	<1–266	43 (88)	<0.1–13	1.6 (3.2)
eel	Rivers – Netherlands	11	6–690	184 (205)	<0.1–1.3	0.3 (0.5)
cormorant (liver)	England	5	138–1320	796 (482)	2.5–14	7.1 (4.5)
common tern (eggs)	Western Scheldt	10	330–7100	1501 (1997)	<2.9	
harbor seal (blubber)	W. Wadden Sea	2	63–2055		<14	
harbor porpoise (blubber)	North Sea	4	440–6800	2945 (2920)	<11	
harbor porpoise (blubber)	N. Sea – E. England	5	<5–1019	312 (422)	0.1–418	83 (187)
		Sewage				
influent	Netherlands	5	<330–3800	954 (1613)	<6.9	
effluent	Netherlands	5	<1–18	4.9 (7.6)	3.1–63	42 (24)
sludge	Netherlands	8	<0.6–1300	175 (455)	2–600	79 (196)
influent	SE England	5	<0.4–29.4	6.3 (13)	<3.9–21.7	7.5 (8)
effluent	SE England	5	<3.9		<3.9	
sludge	SE England	5	531–2683	1401 (814)	15.9–112	59 (41)
sludge	Cork, Ireland	6	153–9120	3322 (3942)	<2.4–192	95 (83)
		Landfill				
leachate water (particulates)	Netherlands	11	2.5–36000	5906 (13026)	<0.3–320	54 (108)
		Sediments				
	Scheldt basin	20	<0.2–950	60 (223)	<0.1–67	5.4 (16)
	Western Scheldt	19	<0.6–99	10 (25)	<0.1–3.2	1 (1)
estuarine + riverine	Netherlands	9	<0.8–9.9	3.2 (3.8)	<0.1–6.9	2.2 (2.2)
estuarine + riverine	England	22	<2.4–1680	199 (364)	<2.4–9750	451 (2077)
	Dublin Bay, Ireland	8	<1.7–12	3.3 (5.2)		

HBCD residues in sludges from the U.K. and Ireland were found to be higher than those quantified from The Netherlands, with U.K. sludges demonstrating a range from 0.5 and 2.7 mg(ΣHBCD)/kg (d.w.). Maximum concentrations of 9.1 mg(ΣHBCD)/kg and 192 μg(TBBPA)/kg were quantified in a secondary treated and dewatered sludge sample from Cork, Ireland. Levels of TBBPA in sludges sampled in this study were similar to those reported in Swedish sewage sludges (*25*). The highest ΣHBCD concentrations that were associated with the particulate phase and found in Dutch STW influents, sludges, and effluents were 3800, 1300 and 18 μg/kg (d.w.), respectively. TBBPA was evident in all of the particulate fractions of Dutch STW final effluents (3 to 63 μg/kg d.w.) and in most sludges with a maximum of 600 μg/kg (d.w.). Release of HBCD from contaminated dust (*6*) may account, in part, for their presence in STWs influents and sludges. In the present study, the HBCD diastereomeric profile in sewage sludges indicated the presence of all three congeners (Table 2) and was quantified at the 10^2 to 10^3 μg/kg (d.w.) levels. In sludge samples acquired from Ireland, γ-HBCD appeared to dominate the congener profiles, and in most samples, α- and β-HBCD were also present in almost equal quantities (see Table 2; 2002/533 and 2002/1066). The accumulation of TBBPA in sewage sludges may be explained by the use of recycled thermal paper in the production of toilet paper in which the former has been shown to contain free TBBPA (*26*).

HBCD and TBBPA in Landfill Leachates. Landfill sites in receipt of waste, including dust material contaminated with BFRs, may also represent point sources for the dissemination of these chemicals to the wider aqueous environment. All of the landfill sites sampled here had received domestic waste only, and prior to discharge, leachates had been treated. The leachate samples taken from three U.K. repositories showed no evidence of either HBCD or TBBPA. Compared with other EU countries, the U.K. has a higher use of HBCD due to more stringent fire safety regulations regarding the flame retardancy of upholstered furniture. It was only in the particulate phase of Dutch landfill leachates that HBCD was found and at a mean concentration of 6 mg/kg (d.w.). One Dutch leachate sample (2002/291; Table 2) yielded an extreme maximum ΣHBCD of 36 mg/kg (d.w.), and γ-HBCD was at least four times greater in concentration than the α-diastereomer. TBBPA was detected at a mean concentration of 54 μg/kg d.w. and varied between <0.3 and 320 μg/kg with a median value of <25 μg/kg.

HBCD and TBBPA Concentrations in River and Estuarine Sediments. Concentration data for sediments have been reported on a dry weight (d.w.) basis and are also presented in Table 1. HBCD was detected in all river and estuarine sediments sampled from the U.K., Belgium, and The Netherlands. With reference to Table 2, the highest measured level of ΣHBCD (1.7 mg/kg) and of TBBPA (9.8 mg/kg) were found in freshwater sediment samples (2001/2586 and 2002/2584) acquired from the River (R) Skerne, northeast England. These samples were taken in the vicinity of a site of BFR manufacture at Newton Aycliffe, County Durham. Residues of BDEs have also been reported from this location (*27*). The Skerne is a tributary of the R. Tees, and both HBCD and TBBPA were also evident in the Tees with respective mean values of 510 and 25 μg/kg. Sediments sampled offshore of the Tees estuary showed an absence of both these BFRs. For sediments analyzed from the R. Clyde, Scotland, a range of 7 to 187 μg(ΣHBCD)/kg was found; TBBPA was not detected at all.

Levels of ΣHBCD in Dutch sediments from the R. Rhine were higher than those found in the R. Meuse (see Table 2), and a range of concentrations from 2.3 to 34 μg/kg reflected the industrial activity that is present in the Rhine basin compared to the catchment area of the Meuse. Concomi-

TABLE 2. Concentrations of HBCD Diastereomers and TBBPA in Selected Aquatic Biota (μg/kg Lipid Weight) and Sediments, Sewage, and Landfill Leachate Samples (μg/kg Dry Weight)

sample type	sample number	location	HBCD diastereomer α-	HBCD diastereomer β-	HBCD diastereomer γ-	ΣHBCD	TBBPA
yellow eel tissue (Scheldt Basin)	2001/1485	Leie, Oeselgem	92	18	41	151	<0.1
	2001/1486	Leie, St.-Martens-Leerne	200	<2.4	62	262	2.1
	2001/1487	Scheldt, Doel, Liefkenshoek	25	<1.5	3.5	29	<0.1
	2001/1489	Scheldt, Oudenaarde	133	127	6.0	266	<0.1
sediments (Scheldt Basin)	2001/1466	Leie, St.-Martens-Leerne	7.4	<0.1	31	38	2.3
	2001/1458	Scheldt, Dutch border	<0.2	<0.2	83	83	0.5
	2001/1465	Scheldt, Oudenaarde	180	60	710	950	24
yellow eel tissue (Dutch rivers)	1999/4169	Waal, Tiel	292	<1.7	67	359	1.0
	1999/4175	Rhine, Lobith	234	<1.9	49	283	0.9
	1999/4181	Hollands Diep	178	<1.9	46	224	<0.1
	1999/4223	Meuse, Eijsden	32	<4.8	<4.8	32	<0.2
	1999/4229	Meuse, Keizersveer	25	<1.8	<1.8	25	<0.1
sediments (Dutch rivers)	2000/1979	Rhine, Lobith	<0.6	3.9	4.2	8.1	1.2
	2000/1981	Hollands Diep	<1.4	<1.4	9.9	9.9	4.0
	2000/35617	Meuse, Eijsden	<0.2	<0.4	<0.2	<0.2	1.6
	2000/35618	Kiezersveer	2.8	<0.4	<0.2	2.8	0.8
sea star (whole tissue)	2001/1236	Tees estuary (NE England)	464	<218	306	769	205
harbor porpoise (blubber; U.K.)	1998/7479	Humber (E England)	331	335	352	1 018	418
	1998/7467	Tyne/Tees (NE England)	375	2.5	6.1	3 84	0.31
cormorant (liver; U.K.)	1999/2615	Monmouthshire (Wales)	1060	147	112	1319	14
	2000/2258	Hampshire (S England)	1022	23	41	1086	2.5
sediments (Western Scheldt)	2000/58	Middelgat (Hoedekenskerke)	<0.2	<0.5	7.7	7.7	1.3
	2000/64	Zandvlietsluis	2.6	<1.8	96	99	0.9
	2000/67	Terneuzen	2.1	0.3	57	59	1.0
	2000/0073	Hoofdplaat (reg. station)	<0.2	<0.2	0.7	0.7	0.3
sediments (U.K. rivers)	2000/2345	Tees (NE England)	59	37	267	363	57
	2000/2352	Tees (NE England)	60	23	212	295	12
	2000/2359	Tees (NE England)	106	111	294	511	9
	2000/2589	Tyne (NE England)	32	16	274	322	5
	2000/2586	Skerne (NE England)	745	396	537	1678	<2.4
	2000/2584	Skerne (NE England)	40	16	118	174	9750
	2000/2758	Humber (E England)	<2.4	<2.4	6	6	<2.4
	2000/3989	Mersey (NW England)	<2.4	22	<2.4	22	<2.4
	2002/989	Clyde (Scotland)	64	31	92	187	<2.4
STW (U.K.) influent[a]	2002/1045	Burnham (SE England)	7.9	13	3.2	24	2.6
secondary sludge	2002/1017	Burnham (SE England)	132	458	666	1256	24
	2002/1021	Chelmsford (SE England)	541	897	1245	2683	88
STW (Ireland) secondary sludge	2002/533	Portlaoise	280	349	604	1233	83
	2002/1066	Cork	2840	2480	3850	9170	185
landfill leachate	2002/266	Netherlands	48	13	52	113	320
	2002/291	Netherlands	7000	<23	29000	36000	43

[a] Dissolved phase (ng/L).

tantly, ΣHBCD concentrations in eels from the Rhine were approximately five times greater than those eels taken from the Meuse. The detection of HBCD in sediments (up to 30 μg/kg) from the R. Rhine at Lobith on the German-Netherlands border suggested that these BFR residues had originated from Germany or further upstream of the Rhine. The impact from textile industries utilizing HBCD in their products and from flame retardant production and processing facilities at Terneuzen (The Netherlands) was evident in sediments from the Western Scheldt and Antwerp harbor on the Dutch border and at several locations within the Scheldt basin in Belgium. Despite its higher production volume, and in comparison to HBCD, TBBPA was detected in some, but not all sediment samples (Table 1), and at substantially lower levels than HBCD.

The HBCD diastereomeric pattern in most sediment samples was similar to that of the commercial formulation (Figure 1) where γ-HBCD predominated and α-HBCD was <10% of the sum of the three isomers. The β-isomer was present in the smallest quantities. The thermal rearrangement of the HBCD diastereomers may occur at temperatures above 160 °C, and such temperatures are utilized in some processes that incorporate HBCD into a polymer matrix. An isomeric rearrangement brings about the conversion of γ-HBCD into the α-diastereoisomer resulting in a considerably higher percentage of α-HBCD in the polymer (*18*). At some locations, e.g., the R. Scheldt near Oudenaarde and St. Martens-Leerne (Belgium), the detection of higher percentages of both the α- and β-isomers may have been indicative of the use of HBCD in polymer and/or textile materials. An absence of α- and γ-HBCD was observed in sediments acquired from the R. Mersey, U.K. (see Table 2; 2000/3989) and the β- congener dominated residue levels in two out of five samples. The deviation from the γ-HBCD dominated profiles that are frequently found in sediments is unusual.

Biomagnification of HBCD and TBBPA Residues in Aquatic Food Webs. Since the rivers investigated in this study eventually drain into the North Sea basin, residue levels of these BFRs were also measured in animals representing different trophic levels from the North Sea food web. There is evidence to suggest that biomagnification of HBCDs is occurring through these webs. Concentrations reported here have been normalized to the lipid weight (l.w.) of the organism, since HBCD has a tendency to accumulate in lipid-rich tissues of the biota. The bioaccumulative potential of HBCD was supported by evidence of biomagnification through the food web of the mysid shrimp via gudgeon, to eggs of the common tern found in the Western Scheldt

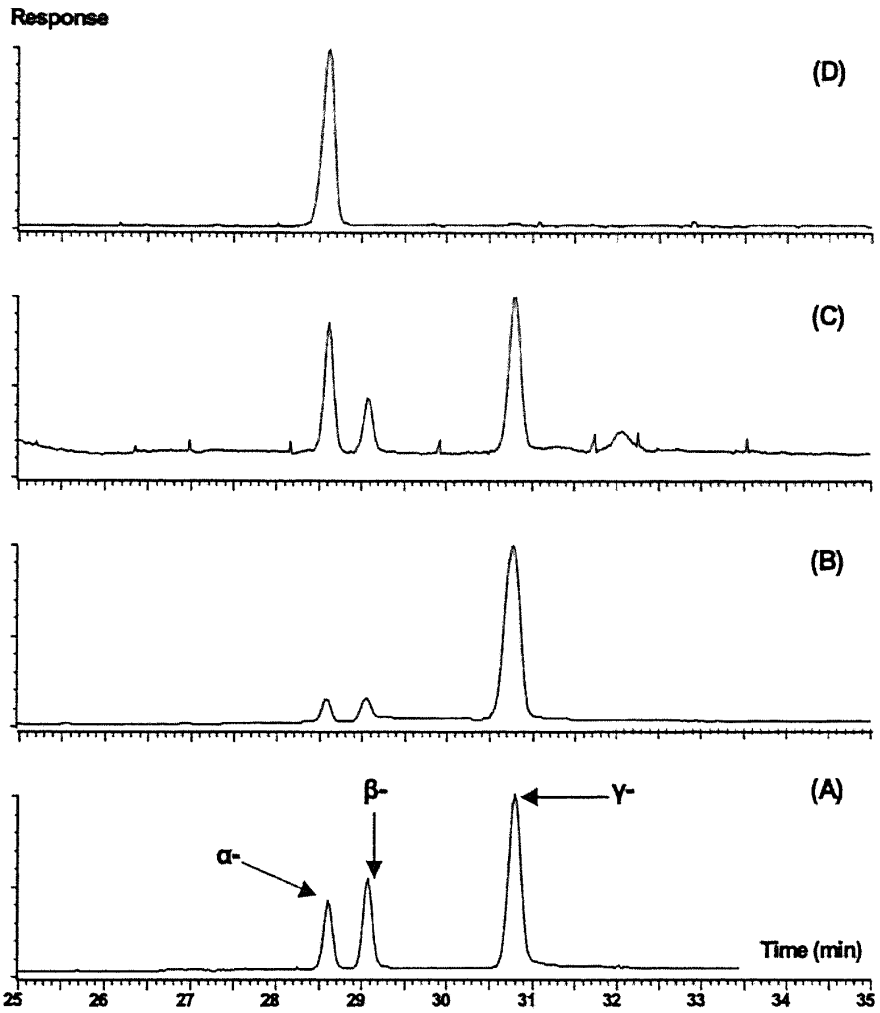

FIGURE 4. LC-MS chromatograms of HBCD diastereomers. (A) HBCD technical formulation, (B) estuarine sediment (NE England; α=60, β=23, γ=212 μg/kg d.w.), (C) sea star (*Asterias rubens*), Tees estuary (NE England) (α=464, β=<218, γ=306 μg/kg l.w.), and (D) harbor porpoise (*Phocoena phocoena*) blubber, Humber estuary, NE England (α=101, β=<1.2, γ=<1.2 μg/kg l.w.).

estuary. Tern eggs showed ΣHBCD residues ranging from 0.3 to 7.1 mg/kg. The biomagnification of ΣHBCD is not a simple process to explain since, at the trophic levels of fish and top-predators, α-HBCD always appears to dominate the diastereomeric profile (Figure 4D). This phenomenon may be caused by a preferential uptake of the α-diastereoisomer, possibly due to a higher water solubility of the α-isomer (40 μg/L) compared to that of γ-HBCD. This would cause a preferential transfer of the α-isomer from particles through the water phase into organisms. Other explanations include a rapid elimination of the β- and γ-diastereomers from the organism, an exposure to α-HBCD, or some other unknown factors. Conversion of one diastereomer into another by a biological system has not been reported.

At the sediment sampling locations of the Scheldt, HBCD residues in yellow eels reflected the spatial distribution of HBCD concentrations found in sediments (Table 2). A maximum ΣHBCD concentration of 44 μg/kg (l.w.) was evident in eels taken from the R. Scheldt at Oudenaarde (2001/1489). A level of a similar order of magnitude was also identified in eels from the R. Leie at Oeselgem (41 μg/kg). Such values are indicative of the bioaccumulation processes that are occurring in the freshwater environment as well as the influence of the production and use of these BFRs in these localities. It is known that a high density of textile industries is located in and upstream of Antwerp harbor, and a number of these may be utilizing HBCD in their products. Manufacture and use of HBCD also occur at Terneuzen, on the border of the Western Scheldt. It is hypothesized that losses of the technical HBCD formulation during manufacture would mainly consist of γ-HBCD, whereas losses from textile or plastic industries incorporating HBCD into their products by heating resins would have diastereomeric profiles dominated by α-HBCD. The congener profiles in eels reflected the observations from other biota analyzed in this study, in that α-HBCD was the dominating congener. It is most likely that the less abundant, β- and γ-congeners were present in these samples but at concentrations below the limits of detection of the applied methodologies. For most eel samples, the α:γ concentration ratio was between 2.5 and 3.5. However, it is interesting to note the presence of β-HBCD in eels acquired from the R. Scheldt at Oudenaarde where an α:β:γ concentration ratio of 21:21:1 was determined. β-HBCD was also detected in eels from the R. Leie, Oeselgem and where an α:β:γ ratio of 5:1:2 was observed. As ΣHBCD levels increase and detection limits are attained, the mutual diastereoiosmeric ratios appear to increase. For most of the other eel samples, β-HBCD was not detected. Eels from two of the three reference sites also demonstrated ΣHBCD concentrations of 210 and 32 μg/kg from the rivers IJzer and Warmbeek, respectively whereas only sediments from the R. IJzer exhibited a measurable, albeit low, trace amount of ΣHBCD (3.2 μg/kg d.w.).

A poor correlation was observed for the spatial distribution of TBBPA residues in eel tissues and those found in sediments from the Scheldt basin. TBBPA in eel samples was generally low and a maximum concentration of 13 μg/kg (l.w.; 2001/1481) was found at the R. IJzer reference site. This concen-

tration was more than three times lower than the maximum ΣHBCD concentration of 44 μg/kg (l.w.). TBBPA was found to be present at high concentrations in only a few sediment samples e.g., 24 μg/kg (d.w.; R. Scheldt, Oudenaarde; 2001/1465) and 67 μg/kg (Beveren, Antwerp; 2001/1470). As a product of its polar nature, it is possible that TBBPA shows less of a bioaccumulative potential than HBCD and is less able to partition to the particulate and organic carbon compartments of sediments. In addition to this, TBBPA may be readily metabolized and, hence, eliminated from the organism.

In this study, marine macroinvertebrates yielded detectable levels of ΣHBCD, albeit 2 orders of magnitude lower than those found in top-predators. A range of <30−84 μg/kg (l.w.) was determined in sea stars from the Western Scheldt and of 29−47 μg/kg in common whelks from the North Sea (Table 1). In a previous study (*23*), the same sea star samples were analyzed for six major BDEs, and it is interesting to note that the tetra-, penta-, and hexa-BDEs were in a similar concentration range. An individual sea star acquired at the mouth of the Tees estuary, U.K. (Table 2; 2001/1236) demonstrated a ΣHBCD of 769 μg/kg, with two diastereomers clearly above the limits of quantification (Figure 4C). This concentration may be indicative of dispersion processes from a point source, such as the HBCD production facility located on a tributary river of the Tees. It is also possible that the tissue concentration may be a product of contaminated food or sedimentary particles located in the intestinal system of the sea star sample and which had been incorporated into the sample matrix prior to extraction.

For marine mammals, the highest levels of ΣHBCD (2.1 to 6.8 mg/kg; Table 1) were found in samples from the West Wadden and North Seas and in samples of liver and blubber. These organisms are lung breathing, top-predators that cannot eliminate their contaminant load to the ambient seawater as efficiently as gill-breathing aquatic organisms. The diastereomeric profiles in harbor porpoises and harbor seals were also strongly dominated by α-HBCD (>80% of ΣHBCD; Figure 4D). Thus, the most abundant isomer detected in marine mammals is also the most thermodynamically stable one found after the HBCD technical product has been subjected to heat during processing. For two individual harbor porpoises, the relative contribution of ΣHBCD in blubber was greater than that of the sum of tetra-hexa-BDEs (*23*). From this previous study, the authors concluded that the R. Tees on the U.K.'s northeast coast is a major source for tri- to hexa-BDEs found in food web organisms. It is also likely that this river system transports BFRs such as HBCDs from a point of manufacture in the Tees catchment area and contributes to their presence in North Sea marine mammals.

Liver samples of a seabird top-predator, the cormorant (*Phalacrocorax carbo*) and which were obtained from the Tees estuary (northeast England), contained ΣHBCD levels in the range of 0.14 to 1.3 mg/kg (l.w.). Again, there was a strong prevalence of α-HBCD (>85% of ΣHBCD). TBBPA was also detected in cormorants from the Tees, although levels were again, approximately one order lower than that of ΣHBCD. For aquatic biota, the relatively low concentrations of TBBPA compared to those of HBCD may be related to the fact that TBBPA is easily metabolized and eliminated from the organism. The presence of the phenolic groups allows direct phase-II biotransformation processes via conjugation to glucuronic or sulfate (*28*). The more polar and reactive molecular properties of TBBPA might result in a lower degree of bioaccumulation. In addition to this, TBBPA may bind to other endogenous compounds such as proteins; these complexes might not be extractable with those extraction techniques applied in this study. Furthermore, in the majority of its uses, TBBPA is chemically bound to the polymer matrix of the product into which it is applied (*10*). In this case, potential emissions of TBBPA from flame retarded products to the environment are likely to be limited in comparison to other BFR compounds such as HBCD and BDEs, which are only mixed with the polymer matrix.

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Supporting Information Available

Details of locations and acquisition dates for samples of aquatic biota and sediments analyzed for TBBPA and HBCD diastereomers (Tables SI-1−5). This material is available free of charge via the Internet at http://pubs.acs.org.

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