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Levels of Hexabromocyclododecane in Harbor Porpoises and Common Dolphins from Western European Seas, with Evidence for Stereoisomer-Specific Biotransformation by Cytochrome P450

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Commercial hexabromocyclododecane (HBCD) is a high-production-volume flame-retardant applied in polystyrene foams. It contains three stereoisomers, of which γ-HBCD always dominates. Here we report on the levels of HBCD in blubber of harbor porpoise and common dolphin from different European seas. The highest total (Σ)-HBCD levels were measured in harbor porpoises stranded on the Irish and Scottish coasts of the Irish Sea (median concentration 2.9 μg (g of lipid)−1) and the northwest coast of Scotland (median concentration 5.1 μg (g of lipid)−1). The median levels in other areas were, for the harbor porpoise south coast of Ireland, 1.2 μg (g of lipid)−1, for the east coast of Scotland (northern North Sea), 0.77 μg (g of lipid)−1, and, for Galicia (Spain), 0.1 μg (g of lipid)−1. The median levels for the common dolphin were, for west coast of Ireland, 0.9 μg (g of lipid)−1, for the French coast of the English Channel between Normandy and Brest, 0.4 μg (g of lipid)−1, and, for Galicia, 0.2 μg (g of lipid)−1. A subset of 10 harbor porpoise and 9 common dolphin blubber samples representing all areas were analyzed by LC/MS to determine the diastereomeric composition of their HBCD residues. All samples showed exclusively the peak of α-HBCD. To test if biotransformation by the cytochrome P450 system could explain the observed compositional difference with technical HBCD mixtures, a number of in vitro assays with microsomal preparations of liver were carried out. We had to revert to material stored at −80 °C from a laboratory rat and a fresh harbor seal found dead in the Dutch Wadden Sea, since such liver samples of cetaceans were not in our possession. The in vitro assays showed that β- and γ-HBCDs were indeed significantly metabolized when incubated in the presence of NADPH as electron donor, compared to a set of reference samples which were identical except for the addition of NADPH. In contrast, the peak of α-HBCD did not decrease significantly in the presence of NADPH. In separate microsomal assays with β- and γ-HBCDs, new peaks of brominated compounds (signal at m/z = 79 or 81) with masses of [M + O] were formed only when NADPH was added. This confirms the process of cytochrome P450 mediated biotransformation. Although rat and harbor seal belong to different families of the mammalia than the cetaceans, we propose that biotransformation by the cytochrome P450 system is also the most likely process to explain the exclusive accumulation of α-HBCD in harbor porpoise and common dolphin.

Introduction

Brominated flame-retardants (BFRs) enhance the fire resistance of many materials. Drawbacks of this great safety feature are environmental problems, since some representatives have become recognized as persistent organic pollutants (POPs) (1–6). By definition, POPs bioaccumulate in lipid-rich tissues of animals and have a high resistance to environmental degradation processes.

Hexabromocyclododecane (HBCD; CAS no. 110-85-0; EINECS no. 203-808-3) is the principal flame-retardant in extruded (XPS) and expanded (EPS) polystyrene foams used mainly as insulation material in the building industry, but is also used in upholstered furniture (7). With a worldwide production of 16700 tons in 2001, it is recognized as a high-production-volume (HPV) chemical and a priority pollutant under the “Existing Substance Regulation” (ESR) of the European Chemicals Bureau (ECB). In Europe and the U.S., HBCD is not subject to regulatory restriction, whereas in Japan it is classified as a type I monitoring substance. HBCD is the third BFR in production volume after the decabromodiphenyl ether mixture (deca-BDE) and tetrabromobisphenol A (TBBP-A). The carbon skeleton of HBCD is formed by one cyclic aliphatic ring of 12 carbon atoms. All mixtures contain the isomers α-, β-, and γ-HBCDs, which are diastereomers, but the γ-isomer always forms the majority (≥70%) (8). Environmental residues of HBCD have been reported recently (9–12). Retrospective analyses of eggs of the guillemot (Uria aalge) from the Baltic Sea demonstrated HBCD residues already in the early 1970s, but the levels started to increase sharply after 1980 (11). Also, the isomeric composition of the residues in biota can deviate substantially from that of the technical mixtures. The two papers that have been published with information on this issue show an increases of α-HBCD with a concurrent decrease of γ-HBCD; this phenomenon seems to become more prominent in vertebrates at higher levels of the food web (9, 12).

In this study, total (Σ)-HBCD levels were measured in blubber of harbor porpoise (Phocoena phocoena) and common dolphin (Delphinus delphis) stranded on the west and east coasts of Scotland, the east, south, and west coasts of...
programs of the different regions. Blubber samples of complete vertical cross-sections were taken from the left side in front of the dorsal fin, following the ECS guidelines for gross postmortem examination tissue sampling (14). Animals sampled ranged in composition state from extremely fresh (2a) to moderately decomposed (3), the numbers referring to the ECS guidelines mentioned above.

**Age Determination of the Animals.** Age was determined on teeth samples collected from the middle of the lower jaw (14).

**GC/MS Analysis of HBCD.** All isomer standards were obtained from Cambridge Isotope Laboratories (CIL). The principal tool for analysis was gas chromatography—negative chemical ionization mass spectrometry (GC—NCI-MS). The gas chromatogram was a Hewlett-Packard 6890 and the mass-selective detector a Hewlett-Packard 5973. GC specifications: split—splitless injection; split valve closed for 1.5 min; T\text{inject} = 250 °C; column, stationary phase CP Sil-8, 30 m × 0.25 mm × 0.25 μm (Varian); carrier gas He; linear gas velocity 74 cm s⁻¹; constant flow programed; oven temperature program, 90 °C (1.5 min)/20 °C min⁻¹/190 °C (0 min)/4.5 °C min⁻¹/270 °C (5 min)/10 °C min⁻¹/320 °C (10 min). MSD specifications: ionization gas CH₄; T\text{ion source} = 210 °C; T\text{transfer line} = 310 °C; T\text{quadrupole} = 160 °C. HBCD was analyzed with selective ion recording at the masses of the two naturally occurring bromine isotopes with m/z = 79 and 81. The methods employed generally follow those described before for the PBDEs, with a minor alteration in the elution of the silica columns employed for cleanup after extraction and sulfuric acid treatment (15, 16); i.e., elution was performed with 30 mL of an 85% pentane/15% diethyl ether mixture.

**LC—MS Analysis of HBCD.** The diastereomeric composition of a selection of blubber samples from the field study and the in vitro assays was studied on the basis of a recently developed LC—MS method with electrospray negative ionization single-quadrupole mass spectrometry (9). The LC—MS system consisted of a Spectrasystems P4000 HPLC pump and an AS3000 autosampler combined with a ThermoQuest Finningan Navigator liquid chromatograph—mass spectrometer. LC conditions: stationary phase; C18 column, 150 × 2.1 mm; particle size 3.5 μm; mobile phase, 0.25 mM ml⁻¹ min⁻¹ 85% methanol/15% 0.1 mM sodium acetate. MS conditions: ionization mode; electrospray negative mode; capillary voltage 4.6 kV; T\text{ionization} = 220 °C. Selected ion monitoring was used, allowing the monitoring of molecules containing bromine isotopes (m/z = 79, 81; source voltage 100 V), the parent HBCD molecule (m/z 640, 642, 644; source voltage 50 V), and monohydroxy-HBCD (m/z 656, 658, 660; source voltage 20 V).

**In vitro Assays with Individual HBCD Isomers.** All assays were carried out in 25 mL Erlenmeyer flasks in a shaking water bath at 37 °C, being the body temperature of the experimental organisms. The flasks contained 1 mL of 0.08 M phosphate buffer (pH 7.6) to which was added 100 μL of microsomal suspension standardized at 10 (mg of total protein) mL⁻¹ (final concentration 1 (mg of total protein) mL⁻¹).

The rat microsomes originated from male 14 week old Wistar rats that had been preinduced with 0.1% w/v phenobarbital in drinking water for 7 days prior to sacrifice (Charles Tiver, Sulzfeld, Germany). These microsomes were a gift from Dr. Ilonka Meerts, Department of Toxicology of Wageningen University (17). For the assays with rat microsomes, the 1:1:1 isomer HBCD mixture consisted of a 200 μM concentration of each isomer in DMSO. A 10 μL sample of this mixture was added to microsomal suspensions of 1 mL (final initial concentration in the incubation mixture, 2 μM per isomer). For the assays with harbor seal microsomes, the stock solutions in DMSO contained 200 μM HBCD, either

**Experimental Section**

**Sampling.** Samples of stranded harbor porpoises and common dolphins were collected as part of the national stranding programs of the different regions. Blubber samples of complete vertical cross-sections were taken from the left side in front of the dorsal fin, following the ECS guidelines for gross postmortem examination tissue sampling (14). Animals sampled ranged in composition state from extremely fresh (2a) to moderately decomposed (3), the numbers referring to the ECS guidelines mentioned above.

**Figure 1.** Map of the sampling areas from which stranded harbor porpoises and common dolphins were analyzed: NNS = northern North Sea (Scottish east coast); SNS = southern North Sea (Dutch/Belgian/French coast north of Calais); NWS = northwest coast of Scotland; IS = Irish Sea (Scottish west coast south of Campbeltown and Irish east coast), SI = Irish south coast (Celtic Sea); WI = Atlantic Irish west coast; WCH = western English Channel between Normandy and Brest; GAL = Galician north and west coasts. No strandings occurred in the eastern English Channel and the Bay of Biscay.

Ireland, the coasts of The Netherlands, Belgium, and France north of Calais (Southern North Sea), the French coast of the western part of the English Channel (Normandy—Bret), the Bay of Biscay, and the north and west coasts of Galicia in Spain (Figure 1). Only female animals were chosen to limit the variation in levels to sex differences. Moreover, female harbor porpoises migrate less than males in these waters, and they are thus more representative for the area (13). The Σ-HBCD analyses were carried out using gas chromatography combined with negative chemical ionization mass spectrometry (GC—NCI-MS) for quantification.

The isomeric composition of the HBCD residues was also determined in a selection of samples. Since the high temperatures in gas chromatography cause rearrangement of the diastereoisomers into each other, the isomer pattern was determined by LC—MS (9). The results of this analyses gave us reasons to test whether differences in the rates of biotransformation by the cytochrome P450 system (CYP450) in liver for the three HBCD diastereomers could explain the biotransformation by the cytochrome P450 system (CYP450)

![Diagram](https://example.com/diagram.png)

**FIGURE 1.** Map of the sampling areas from which stranded harbor porpoises and common dolphins were analyzed: NNS = northern North Sea (Scottish east coast); SNS = southern North Sea (Dutch/Belgian/French coast north of Calais); NWS = northwest coast of Scotland; IS = Irish Sea (Scottish west coast south of Campbeltown and Irish east coast), SI = Irish south coast (Celtic Sea); WI = Atlantic Irish west coast; WCH = western English Channel between Normandy and Brest; GAL = Galician north and west coasts. No strandings occurred in the eastern English Channel and the Bay of Biscay.
FIGURE 2. Box and whisker plots of HBCD levels in µg (g of lipid)\(^{-1}\) in blubber of female harbor porpoise (PP) and common dolphin (DD) stranded along the different European coasts identified in Figure 1. The lower, central, and upper horizontal bars in the boxes show the lower quartile, the median, and the upper quartile of the lipid-normalized concentrations. The error bars indicate the range of all concentrations that were between the inner fences (median ± 1.5(IQD) (the interquartile distance)). The numbers below the species indicate \( n \) the number of animals analyzed for each area.

FIGURE 3. Same for the age distribution of the different animals. The lower, central, and upper horizontal bars in the boxes show the lower quartile, the median, and the upper quartile of the lipid-normalized concentrations. The error bars indicate the range of all concentrations that were between the inner fences (median ± 1.5(IQD) (the interquartile distance)). The numbers below the species indicate \( n \) the number of animals analyzed for each area.

as a single isomer (α, β, or γ) or as a 1:1:1 mixture of a 66 µM concentration of all three isomers. A 3 µL sample was added to the microsomal suspensions of 1 mL. CB153 (150 µM) and BDE49 (9 µM) were also added; CB153 (2,2',4,4',6,6'-hexachlorobiphenyl) was used as an internal standard, being resistant to biotransformation. BDE49 (2,2',4,5',tetra bromodiphenyl ether) was added as a positive control, since it is rapidly metabolized.

After 3 min of preincubation, 100 µL of 11 mM NADPH was added every 10 min as an electron donor for the biochemical reaction to each assay Erlenneyer, but not to the references. After 15, 30, or 90 min of incubation, the flasks were put on ice and 1 mL of ice-cold methanol was added to stop the biochemical reactions. Then 100 µL of 11 mM NADPH, 100 µL of 11 mM NADPH, trimethylpentane, or as a 1:1:1 mixture of a 66 µM concentration of all three isomers. A 3 µL sample was added to the microsomal suspensions of 1 mL. CB153 (150 µM) and BDE49 (9 µM) were also added; CB153 (2,2',4,4',6,6'-hexachlorobiphenyl) was used as an internal standard, being resistant to biotransformation. BDE49 (2,2',4,5',tetra bromodiphenyl ether) was added as a positive control, since it is rapidly metabolized.

After 3 min of preincubation, 100 µL of 11 mM NADPH was added every 10 min as an electron donor for the biochemical reaction to each assay Erlenneyer, but not to the references. After 15, 30, or 90 min of incubation, the flasks were put on ice and 1 mL of ice-cold methanol was added to stop the biochemical reactions. Then 100 µL of 2.2 µM CB112 (2,3,3',5,6-pentachlorobiphenyl) was added as a recovery standard to correct for losses during sample pretreatment and for the GC–MS response. The contents of the flasks were extracted five times with 10 mL of pentane in total. Cleanup procedures with sulfuric acid and silica columns were the same as for GC–MS analysis described above (15, 16). However, for observation of newly formed metabolites, no cleanup steps were performed since they can destroy (sulfuric acid) or retain (silica columns) the newly formed metabolites. For LC–MS analyses, trimethylpentane was exchanged with 100 µL of methanol.

Results and Discussion

HBCD Levels in Harbor Porpoises and Common Dolphins from Different European Seas. HBCD levels in blubber of females of harbor porpoise and common dolphin are shown in box and whisker plots (Figure 2). The \( \Sigma \)HBCD levels in harbor porpoises that stranded on the Scottish and the Irish coasts of the Irish Sea and on the coast of northwest Scotland were significantly higher than the levels in all other areas, except for the south coast of Ireland (SI) (\( P \) from ANOVA on logarithm-transformed HBCD concentrations, \(< 0.001; P < 0.05\) in pairwise Tukey post hoc tests for the different areas) (Figure 2). \( \Sigma \)HBCD levels in common dolphins showed less variation than those in the harbor porpoises, since levels similar to those found in harbor porpoises from the Irish Sea and the northwest coast of Scotland were not found in common dolphins. Common dolphins stranded on the west coast of Ireland, the English Channel between Normandy and Brest, the Bay of Biscay, and coasts of Galicia. Thus, the areas where common dolphins stranded were clearly complementary to the stranding areas for harbor porpoises. The only area where standings of both species occurred was Galicia. Figure 3 shows the box and whisker plots for the age distribution of the stranded females. The common dolphins were generally older than the harbor porpoises; especially the median age of 18 years of the common dolphins stranded in the Western Channel was high. All median ages for the harbor porpoises were below 5 years. The levels of \( \Sigma \)HBCD in the two species from the Galician coasts were very similar too, despite a considerable age difference between the common dolphins and the harbor porpoises. The correlation between age and \( \Sigma \)HBCD levels was not significant for both cetacean species from the different sampling areas (not shown).

Since there is no known HBCD producer near the Irish Sea or in northwest Scotland, industries using commercial HBCD formulations are the most likely sources for this pollution. However, a single large source in the Irish Sea would already suffice to explain our results in the harbor porpoises from the Irish Sea and the northwest coast of Scotland, since measurements on the transport of radioisotopes from the Sellafield nuclear plant have demonstrated that the outflow of the Irish Sea is mainly through the Northern Channel into the Scottish coastal current along the Scottish northwest coast (18–20). The values of the lipid-normalized levels of HBCD within the interquartile distance in Figure 2 for harbor porpoises stranded on the coasts of the Irish Sea and northwest Scotland are of a magnitude similar to that of the levels in pike (Esox lucius) caught just downstream of a textile industry along the Swedish river Viskan (21), and in eel (Anguilla anguilla) and brown trout (Salmo trutta) caught near a sewage treatment plant at Newton Aycliffe along the U.K. river Skerne, to which the sewer outlet of an HBCD production plant was also connected (22). In areas not directly connected to local HBCD sources in Lake Ontario and Scandinavia, the range of levels in several
fish species (12) and bird eggs (11, 23) was similar to our other sampling areas.

**Analysis of Stereoisomers of HBCD with LC–MS.** The high temperatures used in GC–MS analysis prevent the discrimination between the different HBCD isomers, since they change to mainly the thermodynamically most stable R-isomers at temperatures >160 °C (8). Therefore, we also used our recently developed LC–MS method (9) to analyze the isomer composition of the HBCD residue in blubber of a selection of 10 harbor porpoises and 9 common dolphins. All samples contained exclusively the α-isomer. This is a surprising result when the universal dominance of the γ-isomer in all commercial mixtures is taken into account. Thus, commercial HBCD cannot be regarded as an unreactive mixture in the environment, and there are several processes that can contribute to the observed enrichment of the thermodynamically most stable α-isomer at the top of the food web.

In the four replicate preparations of (phenobarbital-induced) rat microsomes with NADPH, the peaks of the β- and γ-isomers of HBCD in an artificial 1:1:1 mixture showed a highly significant decrease, but the peak of the α-isomer had not decreased significantly even after the full 90 min of incubation at 37 °C (Figure 4).

In the incubations of a 1:1:1 mixture of HBCD isomers with harbor seal microsomes, we noticed an average decrease of 69 ± 16% for the β-isomer and of 60 ± 10% for the γ-isomer after 90 min, but also here α-HBCD was not significantly biotransformed after 90 min of incubation, with an average decrease of only 17 ± 14% (n = 4). These decreased rates for β- and γ-HBCD were equivalent to biotransformation rates of 1.52 and 1.32 pmol min⁻¹ (mg of microsomal protein)⁻¹, respectively.

In additional assays with the single β- and γ-HBCD isomers, we also looked for the formation of new peaks with LC–MS (n = 2). When such peaks appear only after the addition of NADPH, they are indicative of metabolites. For
β-HBCD, three such bromine-containing metabolites could be observed in the m/z = 79 and 81 trace, while for γ-HBCD two peaks were observed (Figure 4). All peaks eluted prior to the peak of the parent compound, indicating an increased polarity. The quantities of the metabolites formed were insufficient to obtain their full-scan mass spectra. To maintain a maximum of sensitivity at the cost of some selectivity, we therefore chose selective ion recording at the masses of the supposed molecular ions of the metabolites formed. Since monohydroxy metabolites are the most likely initial reaction products of HBCD biotransformation by the cytochrome P450 system, we assumed that an H atom would be replaced by an –OH group, and thus, a small mass range at 16 mass units above the mass range of the strongest signal of the molecular ions of the parent HBCD isomers was selected for this purpose. The m/z = 655–660 LC–MS trace of β-HBCD showed two peaks with the same retention times as the first and third eluting peaks in the bromine isotope trace (Figure 5, Mα,3 and Mα,5). The second metabolite peak in the bromine ion trace of β-HBCD (Mα,2) did not show such a corresponding peak in the m/z = 655–660 trace, suggesting that this was either not a monohydroxy metabolite or a metabolite without a stable molecular ion.

In the case of γ-HBCD, there was a complete match in retention times between the two new peaks appearing after biotransformation in the bromine isotope trace and those in the 655–660 trace (Figure 5). A peak with a retention time similar to that of Mα,3 in the in vitro experiments was also found in residues in some blubber samples, indicating that at least one of these metabolites may still accumulate to a certain degree in lipid-rich tissues of marine mammals.

This enormous change in composition from the commercial mixtures to the residue in marine mammals probably does not occur in a single step from food to marine mammals, since field surveys have shown that the fraction of the α-isomer of ZHBCD already starts to increase gradually at lower trophic levels of the food webs in lakes (12, 26), and the North Sea (9), but not to the extreme degree that the α-isomer has become the only remaining isomer in the residue, as we have observed in cetaceans.

Possible Relation to Toxic Effects. Although only a few data exist on the toxicity of HBCD, they provide reasons for concern. HBCD induced genetic recombination, which is known to provoke a number of diseases including cancer, in a manner similar to that of PBDEs, PCBs, and DDT (27). Neurotoxic effects have also been reported, since HBCD inhibited plasma membrane uptake of the neurotransmitters dopamine, glutamate, and γ-aminobutyric acid (GABA) at the micromolar level (28). The large differences in bioaccumulation potential among the three different isomers almost certainly influence their individual toxicity, but biotransformation by the cytochrome P450 system may increase as well as decrease the toxicity. Thus, it is too early to say whether most environmental hazard comes from α-HBCD or from the β- and γ-isomers, since the reported effects of HBCD on the thyroid hormone regulation (29) may well be caused by the hydroxylated metabolites, in a manner similar to that reported for the PBDEs and the PCBs (17, 30).

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Supporting Information Available

An Excel file showing detailed sampling information. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


(22) Alchin, C. R.; Morris, S. Hexabromocyclododecane (HBCD) diastereomers and brominated diphenyl ether congener (BDE) residues in edible fish from the rivers Skerne and Tees, U.K. *Organohalogen Compd.* 2003, 61, 41–44.


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