Hexabromocyclododecane Diastereomers and Enantiomers in White-Sided Dolphin Blubber and Liver Tissue

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Introduction

The technical hexabromocyclododecane (HBCD) mixture consists primarily of γ-HBCD (~80%) with smaller amounts of α-HBCD (~6%), β-HBCD(~8%), and lesser brominated compounds (~6%).[1] HBCD is used as a flame retardant, primarily in expanded polystyrene foams. In 2001 the worldwide use of HBCD was 16700 tons with 57% of the use in Europe and 17% in the U.S. HBCD has been shown to bioaccumulate[1-5] and biomagnify[2] in aquatic ecosystems. These compounds have also been measured in egg of peregrine falcons[6] and guillemot[7] and in surficial sediment.[3, 4] Concentrations in harbor porpoise and common dolphin blubber ranged from 51 ng/g lipid to 9600 ng/g lipid.[8] While γ-HBCD is the predominant isomer in the technical mixture, α-HBCD is found in higher concentrations in biota. The objective of this work was to measure the concentrations of the three HBCD isomers in specimen banked Atlantic white-sided dolphin (*Lagenorhynchus acutus*) blubber and liver and determine the enantiomeric distribution among animals and within blubber and liver.

Materials and Methods

Sample Extraction and Preparation

Blubber (*n* = 47) and liver (*n* = 6) samples from dolphins were obtained from the National Biomonitoring Specimen Bank (NBSB) maintained by National Institute of Standards and Technology (NIST). All samples were collected on the Massachusetts coast between 1993 and 2000. Most animals stranded in good condition. Prior to extraction, $^{13}C_{12}$-α-HBCD, $^{13}C_{12}$-β-HBCD, and $^{13}C_{12}$-γ-HBCD were added as internal standards. Each tissue sample (~0.5 g blubber or ~3 g liver) was mixed with ~30 g Na_2SO_4 and extracted with dichloromethane using pressurized fluid extraction. Following extraction, lipids and other matrix interferences were removed by gel permeation chromatography (GPC) and solid phase extraction using 5% deactivated alumina. Prior to GPC, a sub-sample of each liver extract was used for gravimetric lipid content determination.

Determination of Diastereomer Concentrations

An Agilent 1100 series liquid chromatography system coupled to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) was used for all analyses. The column temperature was 27 ºC, the autosampler temperature was 20 ºC, and 10 mL injections were used.

The liquid chromatography method was based on a previously described method.[5] Separation of the three HBCD isomers was performed using a C18 column (Agilent ZORBAX Eclipse XDB; 250 mm x 5 mm; 5 mm) and a tri-solvent system: methanol, acetonitrile, and water. A constant 300 mL/min flow rate was used. The initial solvent composition was 60% water/30% methanol/10% acetonitrile. The solvent mixture was changed linearly over 4 min to a final solvent composition of 50% methanol/50% acetonitrile.

Determination of Enantiomeric Fractions

The method for separation of the six HBCD stereoisomers was based on a previously described method.[5] A cyclodextrin column (PhenomonexNucleosilb-PM, 200 mm x 4 mm) and a solvent system consisting of water, methanol, and acetonitrile were used. A constant 500 mL/min flow rate was used. The initial solvent composition was 49% water/30% methanol/21% acetonitrile. The solvent mixture was changed linearly over 4 min to a final solvent

composition of 30% methanol/70% acetonitrile.

The chiral signature resulting from this analysis was described by the enantiomeric fraction (EF).[9] The EF for each enantiomer pair was determined from the area of each enantiomer in the chiral analysis (Equation 1); $Area_1$ and $Area_2$ are the areas of the first and second enantiomer of each diastereomer to elute.

Equation 1 $$EF = \frac{Area_1}{Area_1 + Area_2}$$

Results and Discussion

HBCD Concentrations in Blubber and Liver

α-HBCD was found in all blubber and liver samples. β-HBCD and γ-HBCD were below the limit of detection. The α-HBCD concentration in blubber ranged from 14.4 ng/g wet mass to 283 ng/g wet mass with a median concentration of 80.8 ng/g wet mass. The enantiomeric fractions (EF) in blubber ranged from 0.42 to 0.74 with a median of 0.61.

α-HBCD blubber concentrations are correlated with concentrations of PBDEs and PCBs previously measured in these same animals.[10, 11] Figures 1 and 2 compare ∑PBDE and ∑PCB concentrations to the α-HBCD concentration. ∑PCB (r^2 = 0.70, p<0.001) is more strongly correlated to α-HBCD than and ∑PBDE (r^2 = 0.074, p=0.064).

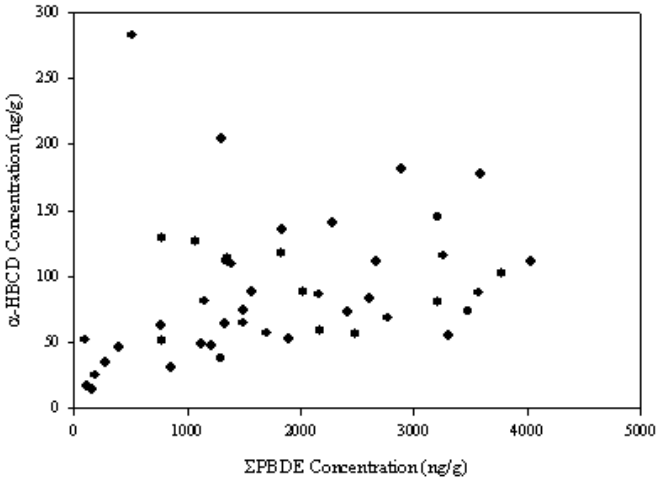

Figure 1. Comparison of ∑PBDE to α-HBCD in white-sided dolphin blubber.

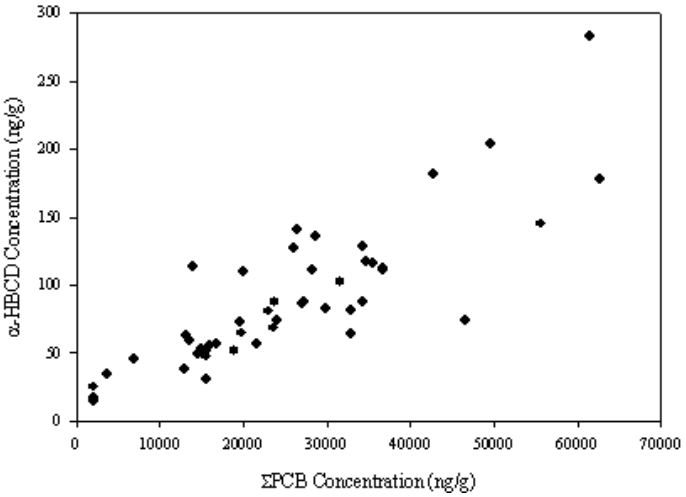

Figure 2. Comparison of ∑PCB to α-HBCD in white-sided dolphin blubber.

The α-HBCD concentration in liver ranged from 2.92 ng/g lipid to 68.3 ng/g lipid with a median concentration of 18.1 ng/g lipid. The EF in liver ranged from 0.42 to 0.62 with a median of 0.55. A comparison of liver and blubber concentrations measured in the same animal is shown in Figure 3.

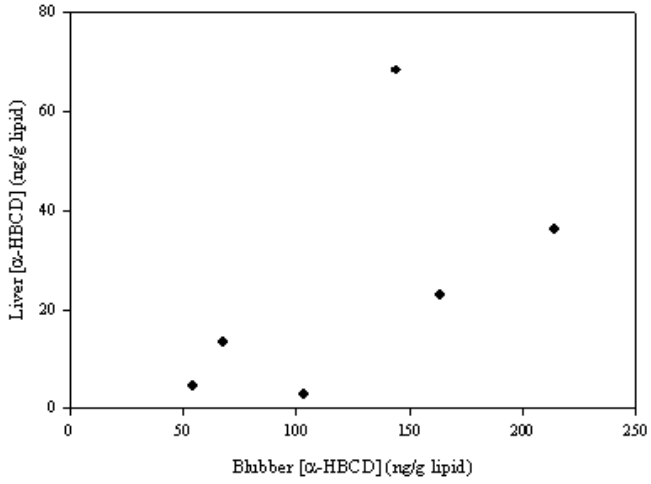

Figure 3. Comparison of α-HBCD in white-sided dolphin blubber and liver.

The HBCD concentrations measured in blubber from white-sided dolphin stranded off the northeastern U.S. coast are on the low end of the concentration range reported for harbor porpoise and common dolphin in European coastal waters, although the α-HBCD isomer dominated in all three cetacean species. These differences are likely due to the larger use of these compounds in Europe than in the U.S. Differences in metabolic capabilities and food chain exposures between species may also be important. Further work is needed to identify the processes controlling HBCD concentrations in marine mammals.

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