Evidence of *bioisomerization* of α– and γ–hexabromocyclododecane (HBCD) isomers in fish

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INTRODUCTION

Hexabromocyclododecane (HBCD) has been used in the last two decades in different products as an additive brominated flame retardant, its main use is in polystyrene foams and resins (1;2). Being an additive flame retardant, HBCD can leak out into the environment during production of the chemical, manufacturing of the final product and disposal of the product. It has been found to have similar physio-chemical properties as other persistent organic pollutants, therefore this leads to the assumption that HBCD may be persistent, bioaccumulative and toxic (3).

HBCD consists of three isomers; α–, β– and γ–. In the technical mixtures, the ratios of each isomer depend on how HBCD is produced, but the approximate ratios of each isomer are 6, 8, and 80% for α–, β– and γ–isomers, respectively(4). The γ–isomer always has the highest percentage and lies between 72-89% (5). Initial reports suggest that the ratios of the isomers in environmental samples are different to do that found in the technical product (6). A recent study done by Tomy *et al*. on the Lake Ontario food web, found that the α–isomer was dominant in pelagic fish; the γ–isomer was consistently lower than the α–isomer while the β–isomer was below detection limits in the samples (7). Even though the β–isomer is present in the technical mixture, it is the least significant in the environment as it is rarely detected.

Selective biotransformation of HBCD isomers can partly explain differences observed in the isomer distribution in environmental samples. This study tests this hypothesis by exposing (both *in vivo* and *in vitro*) juvenile rainbow trout (*Oncorhynchus Mykiss*) to each isomer separately. This enabled us to follow the biotransformation process and screen exposed samples for possible debromination metabolites and to test whether fish can isomerize (*bioisomerize*) individual HBCD isomers.

MATERIALS AND METHODS

Chemicals. Native α-, β- and γ- HBCD, deuterium labelled α-, β- and γ–HBCD and carbon-13–α-, β- and γ–HBCD labelled isomers were provided by Wellington Laboratories Inc. (Guelph, ON, Canada). The purity of all standards was greater than 98%. Methanol and Water, both Optima grade, were obtained from Fisher Scientific (Nepean, ON, Canada).

In-vitro study- incubation of liver microsomes. 890 µL of 0.05M Tris-HCl (hydrochloric acid) buffer with 0.1M sodium chloride (at a pH of 7.0 at 25°C) was added to the micro-centrifuge tube. This solution was made by combining 7.02 g of Tris-HCl (hydrochloric acid), 0.67 g of Tris-base and 5.84 g of sodium chloride and diluting it to 1 L. 100 µL of NADPH in Tris-HCl buffer was added, which was made by adding 100 mg of NADPH in 10 mL of Tris-HCl buffer. 10 ng of HBCD isomer was added (10 µL of 1 ng/µL solution). Each isomer was added to a separate tube and done in triplicate. 100 µL of rainbow trout liver microsomes, held at -80°C were added to the incubation vial after they were thawed on ice. Three sets of blanks were done, one set contained no HBCD, one set contained no NADPH, and one contained no microsomes. The content that was not added to the tubes was replaced in volume by Tris-HCl buffer.

All tubes were incubated at a temperature of 8 ± 1°C in the dark. Each reaction was terminated at time points of 1, 2, 8, 18, and 30 hours, by adding 0.5 mL of HPLC grade methanol and vortexing the micro-centrifuge tube for 1 minute at high speed. Blanks were obtained and quenched at eight hours. The tubes are then stored in the fridge until extraction.

In-vitro study- extraction of HBCD. A 5 µL spike of a 1 ng/µL solution of $^{13}C_{12}$ labelled HBCD, which contained all three isomers, was added to each micro-centrifuge tube as a recovery internal recovery. The sample in the micro-centrifuge tube was transferred into a 10mL graduated test tube by a glass pipette. Then a 1mL aliquot of a saturated salt solution was added to the graduated test tube. The micro-centrifuge tube was rinsed with hexane and transferred into the test tube. Extraction was achieved with three 5 mL rinses of hexane. After each 5 mL rinse, the sample was vortexed for 30 seconds and the organic layer is pipetted into a 15 mL graduated test tube. The sample was then concentrated in 2-propanol to a final volume of 100 µL. 5µL of d_{18} HBCD, a 1 ng/µL solution which contained all three isomers, was added to the sample as an instrument performance internal standard. The analysis was then done by high performance liquid chromatography coupled to tandem mass spectroscopy.

In-vivo study- dietary accumulation of HBCD isomers. Experimental design for this study can be found in Law *et al*. described in these conference proceedings.

LC/MS/MS. HBCD isomers were detected according to the method of Budakowski and Tomy (8). Characteristic [M–H–Br_x, x = 2–4] ions for debromination metabolites were added to the method.

RESULTS AND DISSCUSION

Chromatograms corresponding to day 56 of the depuration phase of the *in vivo* experiment are shown in Figure 1. It should be noted that HBCD was detected in control fish samples, *i.e.*, fish exposed to un-spiked HBCD food. Table 1 shows the control corrected concentrations of each isomer at day 56 of the exposure experiment.

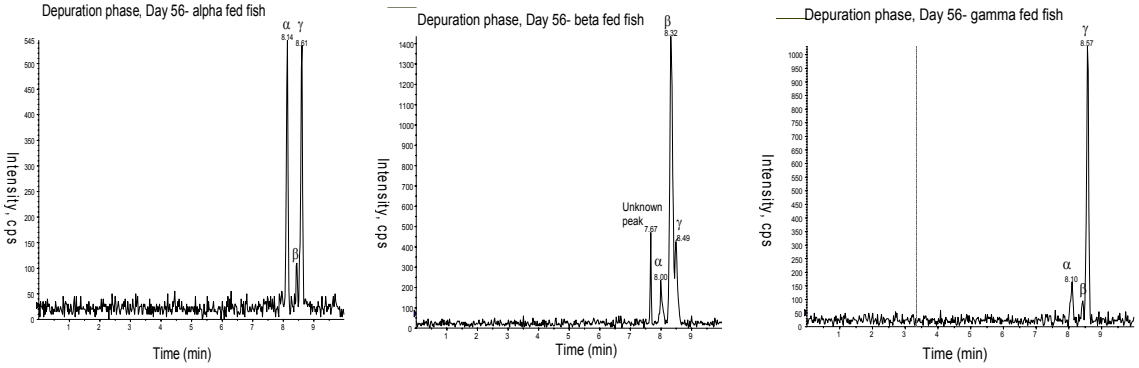

Figure 1. LC/MS/MS chromatograms corresponding to juvenile rainbow trout at day 56 of the depuration phase following dietary exposure to HBCD isomers

It is clear from the results shown in Table 1 that bioformation or *bioisomerization* of HBCD isomers is occurring. Significant concentrations (control corrected) of the β– and γ–isomers were detected in fish exposed exclusively to the α–isomer. Based on a roughly 30-fold difference in concentration, it appears that *bioisomerization* to the γ–isomer from the α–isomer is heavily favoured over similar conversion to the β–isomer. Both the α– and β–isomers are bioforming in fish exposed to the γ–isomer; similar to the results of the α– isomer treatment experiment, the β–isomer is least-favoured. Although data are not presented here, the results of our *in vitro* study are consistent with that of our *in vivo* experiment. It is unclear what enzyme system(s) may be responsible for these observations.

Table 1. Control corrected concentrations (ng/g, lipid wt) of HBCD isomers in muscle tissue of exposed juvenile rainbow trout[a].

	α–	β–	γ–
α –treatment		0.14	3.96
β –treatment	<MDL[1]		<MDL[2]
γ–treatment	7.11	1.57	

[a]Results are for the *in vivo* experiment. There were three treatment tanks and fish in each tank were exposed to one of the isomers. A fourth tank holding 'control' fish were exposed to 'clean' food.

Samples were also screened for possible debromination metabolites. Debromination of brominated diphenyl ethers (BDEs) has already been reported in the literature (9). No

lower brominated cyclododecane compounds were detected in any of the *in vivo* or *in vitro* samples (day 56). A similar result was also found when we screened lake trout samples from Lake Ontario. This should be treated with some caution as if debrominated metabolites are present at low levels, their ion signal may be suppressed because of the matrix.

CONCLUSION

To our knowledge, this is the first report of *bioisomerization* of an organic pollutant in fish. In addition to the many other factors that control the uptake and distribution of persistent organic pollutants in fish, our findings suggest the bioformation will play a critical role in the isomer distribution of HBCD in fish. These results also help to explain differences in HBCD profiles in fish, compared to those in abiotic matrices.

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