Dietary accumulation of hexabromocyclododecane isomers in juvenile rainbow trout (*Oncorhynchus Mykiss*)

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INTRODUCTION

Hexabromocyclododecane (HBCD) is an additive brominated flame retardant. Its use has been increasing within the last twenty years, especially in Europe where HBCD has replaced some of the applications of polybrominated diphenyl ethers(*1*). HBCD is an additive flame retardant, therefore it is not chemically bonded to the polymer and it can leak out into the environment during production, use and disposal of the product (*3*). HBCD has been found to be persistent, and bioaccumulative (*1*).

HBCD consists of three isomers; alpha, beta and gamma (*see* Figure 1). There is little information on the individual isomers in the literature as in the past it has been difficult to separate the isomers using gas or liquid chromatography. Using high-performance liquid chromatography/electrospray tandem mass spectrometry, it is now possible to separate the individual isomers using a method by Budakowski and Tomy (*2*).

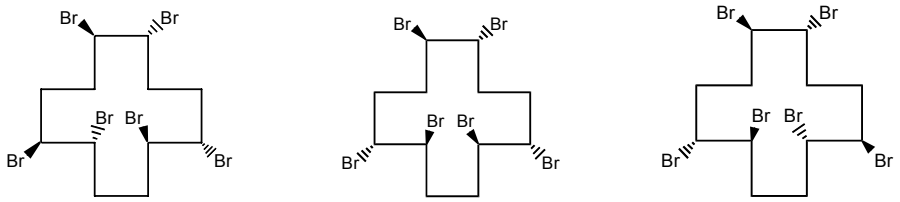

Figure 1. Structures of the α–, RR,SR,RS (left), β–, RR,SR,SR (middle) and γ–, RR,RS,SR (right) HBCD isomers

The goal of this study is to examine the bioaccumulation parameters [depuration rates (k_d), half life ($t_{1/2}$) and biomagnification factor (BMF)] of individual HBCD isomers in juvenile rainbow trout (*Oncorhynchus Mykiss)*. This was achieved by exposing juvenile rainbow trout to environmental relevant doses of the three isomers *via* the diet. A depuration period during which all fish were fed unspiked food was also included to examine elimination. The data presented here are preliminary results of the exposure experiment.

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).

Fish Food preparation. There were four different batches of food that were prepared at different times; the batches were control, alpha, beta and gamma food. The food was prepared by mixing 1.8 kg of clean food with 20 mL of spiked corn oil in a Hobart mixer for 20 mins. The corn oil was spiked separately with ~ 9 ng of each isomer. A premixed binder solution, 40 g of gelatine stirred into 1.5 L of distilled water was added to the food and mixed in the Hobart blender until there was a firm consistency. Noodles were extruded using a meat grinder and then they were dried in an environmental control room for 2-3 days. The noodles were then pellatized and the fish food was stored in a freezer at -4°C until it was needed. The control food was made as above, but no HBCD was spiked into the corn oil.

Fish exposure and sampling days. Juvenile rainbow trout (129 fish in total) were randomly separated into four 800 L round polyethylene tanks. The tanks were kept at a constant water flow rate of 1.4-1.6 L/min, with a temperature of 11-12°C, and a pH between 7.9 and 8.1. The dissolved oxygen was always at a level of saturation. All fish were fed control food for ten days to allow then to adjust to their new surroundings.

Each tank of fish was fed a different isomer fortified food. One tank was fed the control food. Fish were fed 1% of the average total body weight of the tank every second day. The amount of food was adjusted after each sampling day. The spiked or control food was fed to the fish for 56 days for an uptake phase, after this time all tanks were fed control food for 112 days for a depuration phase.

Muscle tissue of four fish from each treatment tank were sampled on days 0, 7, 14, 56 of the uptake phase and days 0, 7, 14, 56, and 112 of the depuration phase. Tissue was kept at -4°C until extraction, clean up and analysis was to be done.

Extraction and Clean up. The muscle tissue was first dry ice homogenized, and then by an accelerated solvent extractor (ASE). In a 33 mL ASE cell 15 g of homogenized muscle tissue, 10 g of diatomaceous earth (heat treated at 660°C for 6 hours), and topped up with Ottawa Sand (heat treated at 600°C for 6 hours) to fill any voids. A 10 µL spike of a 500 pg/µL $^{13}C_{12}$ labelled internal recovery standard that contained all three isomers of HBCD was added to the ASE cell. Sodium sulphate was added to the extract to remove any water. A portion of the extract was then air dried to determine lipid content gravimetrically. The extract was then run through a gel permeation chromatography column to remove the majority of the lipid from the sample. A total of 350 mL of 50:50 hexane: dichloromethane was run through the column. The first fraction was discarded

(150 mL) as it contained the lipid and the second fraction was kept (150-350 mL) as this contained HBCD. The sample was then run through a Florisil column as the final stage of clean up. Each Florisil column contained 8 g of 1.2% deactivated Florisil. Fraction 1, consisting of 35mL of hexane was discarded and fraction 2, 36mL of 15:85 Hexane: dichloromethane and then 50mL of 50:50 hexane: dichloromethane, contained the HBCD. The extract is concentrated to a final volume of 200 µL in 2-propanol, and a spike of 10 µL of a 500 pg/µL solution of d_{18} HBCD containing all three isomers was added to act as an instrument performance internal standard. The analysis was done by liquid chromatography coupled to tandem mass spectroscopy so that near baseline resolution of all three congeners was obtained.

Liquid Chromatography. An Agilent 1100 Series (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser, binary pump and autosampler was used. A Genesis (Jones Chromatography, Chromatographic Specialties Inc.) C_{18} (10.0 cm × 2.1 mm i.d., 4 μm particle size) analytical column was used for all samples. A mobile phase of (A) water and (B) methanol at a flow-rate of 300 μL/min was used. The elution program started at an initial composition of 30:70 (v/v) and was ramped to 100% methanol in 3 minutes. Thereafter it was held for 3.5 minutes and returned to the starting conditions in 3 minutes. The column was allowed to equilibrate for 6 minutes between runs.

Mass Spectrometry. A Sciex API 2000 triple quadrapole mass spectrometer (ABS, Foster City, CA, USA) was used to run negative mode electrospray ionization. The collision cell was recently upgrade by mSpec (ON, Canada) to improve the instrument sensitivity in the multiple reaction mode (MRM). Further details of the MS/MS operating parameters can be found in Budakowski and Tomy (*2*)**.** Quantitation was based on the ion signal from the *m/z* 640.6 → *m/z* 79 MRM transition. MS/MS detection of d_{18}–HBCD and $^{13}C_{12}$–HBCD isomers was based on the analogous ($[M - H]^-$) → Br^- reaction monitored for the native HBCD (*m/z* d_{18}: 657.6 and $^{13}C_{12}$: 652.4 ($[M - H]^-$)).

RESULTS AND DISCUSSION

Figure 2 presents the depuration of α– and β–HBCD in juvenile rainbow trout. Work on the γ–isomer is ongoing. The depuration of the α–isomer appears to occur more rapidly than the β–isomer. The depuration rate, k_d, determined from the slope of a plot of the natural log transformed concentration at each time point (corrected for lipid content) versus time, for the α–isomer ($0.0051 d^{-1}$) was 2.5 × higher than that of the β–isomer ($0.0132\ d^{-1}$).

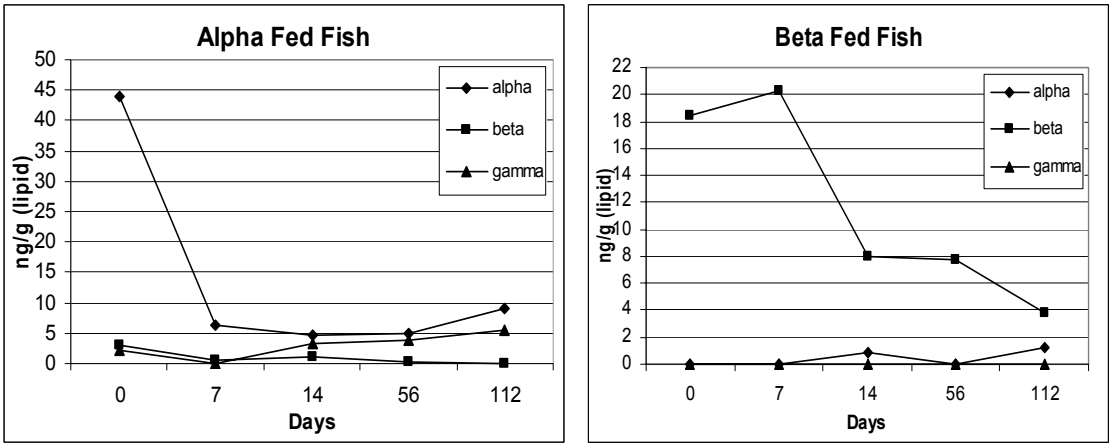

Figure 2: Depuration of α– and β–HBCD in juvenile rainbow trout. Concentrations for the muscle tissue are based on a mean of two fish samples and were control corrected and adjusted for lipid content.

Depuration of both isomers followed a first-order process although the α–isomer appears to depurate rapidly in the first 7 days. Half lives ($t_{1/2} = \ln 2/k_d$), for the α– and β–isomers were 135.9 days and 52.5 days respectively. Compared to PBDEs, the $t_{1/2}$ of the α–isomer falls within the range of the hexa-brominated BDE congeners 153 (115 ± 38 days) and 154 (173 ± 58 days) (*4*). The $t_{1/2}$ of the β–isomer is similar to that found for BDE–138 of 58 ± 14 days (*4*). These comparisons need to be treated with caution as different sizes and fish species were used in the exposure experiments.

CONCLUSIONS

These preliminary results suggest that there are differences in the $t_{1/2}$'s of α– and β–HBCD and these differences are likely to play a role in the isomer distribution and dynamics of these compounds in biota. Work is continuing on measuring concentrations of the three isomers in the muscle tissue during the uptake phase of the experiment.

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