In Vivo and In Vitro Debromination of Decabromodiphenyl Ether (BDE 209) by Juvenile Rainbow Trout

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Introduction

Decabromodiphenyl ether (BDE 209) is the major component of the commercial flame retardant mixture known as decaBDE. This mixture is commonly used to flame retard polymers such as high impact polystyrene found in TV and computer casings. High concentrations of BDE 209 have been reported in sediments, biosolids and house dust [1-3]. In the environment, BDE 209 is susceptible to degradation, via debromination, following exposure to UV light [4]. Dietary exposure studies with carp and rainbow trout have also shown that BDE 209 can be debrominated by apparent metabolic routes [5,6]. The present study was undertaken to examine the in vivo uptake and any biotransformation of BDE 209 by juvenile trout along the gut wall that would lead to accumulation in other fish tissues. These results will be used to calculate the uptake and assimilation rates of BDE 209 in the parent form and all observed debrominated products. Histological examinations of the gonadal and liver tissues are being performed to assess any damage and disruption caused by the exposure on a cellular level. In addition, liver and intestinal microsomes are being prepared and dosed with BDE 209 to assess enzymatic-mediated debromination in vitro. The results from this study will be compared with a previous study using common carp[5] to compare the debromination products observed between the two fish species.

Materials and Methods

Fish Exposure. Juvenile rainbow trout (ave. weight 91.2 ± 5.8 g) were reared at the U.S. Department of Agriculture's National Center for Cool and Coldwater Aquaculture in Kearneysville, WV. Sixty-two fish were randomly separated into four square polyethylene flow-through tanks. Fish in all tanks were acclimated to the control diet (non-spiked food) for a period of approximately three weeks prior to the start of the exposure in which experimental fish were fed BDE 209 amended food. One tank was fed a control diet throughout the experiment and the remaining three tanks were fed food spiked with decabromodiphenyl ether (BDE 209) at a concentration of 944 ± 14 ng/g wet weight. During the five month study period, fish were fed spiked or control food at a rate of 1% of their body weight/day, Monday through Friday, of each week. This resulted in 112 exposure-feeding days. One fish from each tank was sampled on nine different time points throughout the five month exposure. Fish were sacrificed approximately two to three hours post feeding and dissected to remove the gut and liver tissues. Residual food inside the stomach and the gut tissue were also stored for separate analyses. Blood samples were drawn at four different time points throughout the exposure period and will be measured for PBDEs and any hydroxylatedPBDEs. The remaining fish carcass was homogenized and stored at -20 °C prior to analysis for PBDEs.

Sample Extraction. All PBDE analyte standards used for quantification were purchased from either Accustandard (New Haven, CT), Cambridge Isotope Laboratories in Andover, MA or Wellington Laboratories in Guelph, Ontario (Canada). A ^{13}C labeled chlorinated diphenyl ether (2,2',3,4,5-pentachlorodiphenyl ether) was used as an internal standard to quantify the tri- through octaBDE congeners while ^{13}C labeled 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE 209L) was used as an internal standard for the quantification of the three nonaBDEs and BDE 209. All solvents used were HPLC-grade. Pressurized fluid extraction with dichloromethane was used to extract the tissues. The fish extracts were concentrated to 1.0 mL and injected onto an HPLC for size exclusion chromatography to remove lipids. Extracts were injected into the HPLC and eluted through a divinylbenzenepolystyrene column (10 µm particle size, 100 Å pore size, 2.5 cm i.d. x 60 cm, PL-Gel, Polymer Labs, Inc., Amherst, MA) at a flow rate of 10 mL/min of dichloromethane. The collected fraction was reduced in volume and transferred to 0.5 mL hexane. Extracts were then eluted through silica solid phase extraction cartridges. Cartridges were pre-cleaned with 10 mL of hexane and eluted with 20 mL of hexane. The final extract was reduced in volume to 0.5mL in hexane for analysis of PBDEs.

Instrumental Analysis. Extracts were analyzed for PBDEs using gas chromatography (GC) coupled to a mass spectrometer operated in electron capture negative ionization (GC/ECNI-MS). A 0.25 mm x 15 m fused silica capillary column coated with a 5% phenyl methylpolysiloxane (0.25 Fm film thickness) was used for the separation of PBDE congeners. On column injection was employed in the GC, and the injection port was set to track the oven temperature. The oven temperature program was held at 80 EC for 2 min followed by a temperature ramp of 12 EC/min to 140 EC, followed by a temperature ramp of 5EC/min to a final temperature of 280 EC which was held for an additional 20 min. The transfer line temperature was maintained at 280 EC.

Results and Discussion

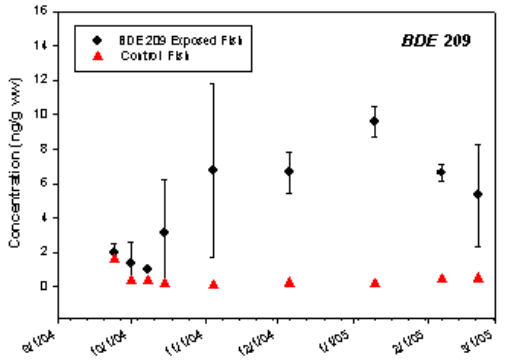

Figure 1. Concentration of BDE 209 measured in control and exposed fish during the experiment.

Low background levels of BDE 209 were measured in all rainbow trout samples from the control group and ranged in concentration from 1.6 ng/g wet weight to 0.1 ng/g wet weight (Figure 1). The concentration of BDE 209 in these control samples decreased throughout the first 30 days of the experiment and then remained at a concentration of approximately 0.2 ng/g wet weight for the remainder of the experiment. In contrast, the concentration of BDE 209 increased in the rainbow trout which were exposed to dietary BDE 209 at a rate of 9.4 µg/kg body weight/day (Figure 1). The mean concentration of BDE 209 from the three replicate exposure tanks increased throughout the first four months of exposure from 1.4 ng/g wet weight to 9.6 ng/g wet weight. However, during the last 1.5 months of the exposure the concentration of BDE 209 decreased in whole fish tissues from 9.6 ng/g wet weight to 5.3 ng/g wet weight. It is not clear at this time why the concentration of BDE 209 decreased during this latter part of the exposure. It is possible that either the trout experienced a physiological change during the month of January that resulted in a reduction in their metabolism, or specific enzymes were induced during this time period that resulted in elevated biotransformation of BDE 209. More work is being conducted to determine the activity of cytochrome P450 and deiodinase enzymes in the liver tissues for indications of enzyme alterations.

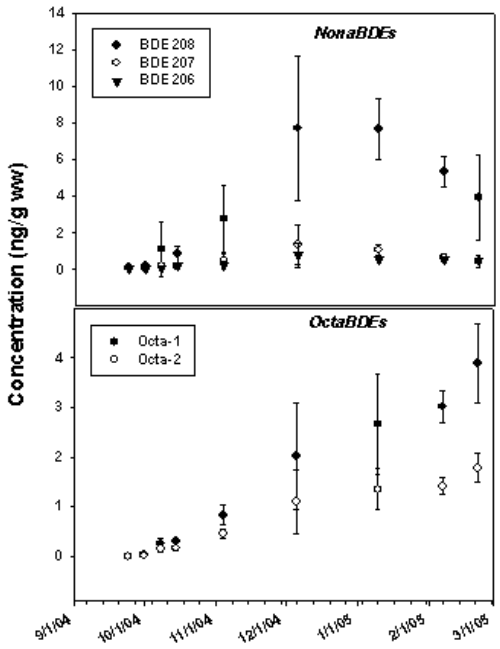

Figure 2. Concentration of Nona- and Octa BDEs measured in whole tissues of Rainbow Trout exposed to dietary BDE 209 for 5 months.

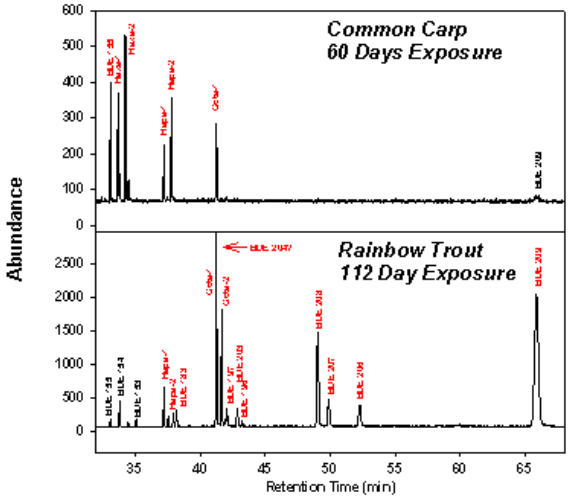

Figure 3. A comparison of GC/ECNI-MS chromatograms of common carp (A) and rainbow trout (B) whole tissue extracts highlighting the different BDE congeners detected. Both fish had received dietary exposure to BDE 209.

NonaBDEs were measured in trout tissues after one week of exposure and octaBDEs were observed in the second week of exposure (Figure 2). Concentrations of BDE 208 increased significantly throughout the experiment and were very similar to the levels and temporal pattern of BDE 209. In contrast, octaBDE congeners increased throughout the entire exposure period. Five octaBDE congeners, including BDE 196, BDE 197 and BDE 203 were observed to increase in concentration throughout the dietary exposure to BDE 209. Four heptaBDE congeners, including BDE 183, also increased in concentration throughout the exposure period. Only one hexaBDE congener, BDE 154, appeared to increase in concentration throughout this experiment. This pattern is very similar to the observations made by Kierkegaard et al. [6]. In a previous study common carp were exposed to BDE 209 amended food for a period of 60 days[5]. The whole fish tissue extracts from the carp exposed to BDE 209 for sixty days were re-analyzed by GC/ECNI-MS during this study to compare to the rainbow trout tissues. As seen in Figure 3, the same prominent octaBDE congener was observed in both fish species following exposure to BDE 209. In addition, two of the same heptaBDE congeners were also observed in both fish. The striking difference between the two fish was the presence of the three nonaBDE congeners in the rainbow trout that were absent in common carp. These differences in the accumulation pattern of debrominated metabolites may suggest that carp have an enhanced ability to metabolize BDE 209 and other BDE congeners relative to rainbow trout. Previous work on carp has demonstrated that carp can debrominate BDE 99 and BDE 183 rapidly, resulting in little to no accumulation of these compounds in their tissues[7].

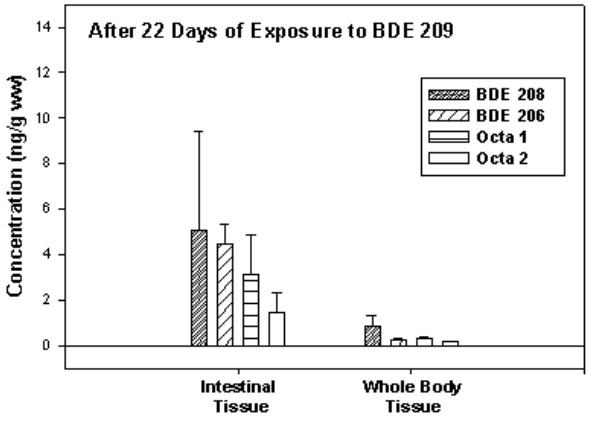

Figure 4. Concentrations of nona BDEs and octa BDEs detected in intestinal and whole body tissues of rainbow trout on Day 22 of the dietary exposure.

Results from the first month of the present experiment demonstrate that the concentration of PBDEs was consistently higher in the intestinal and gut tissues relative to the carcass of the rainbow trout. Concentrations were 5 to 20 times higher in intestinal tissue relative to the whole body tissue (Figure 4). BDE 209 and minor levels of nonaBDEs were detected in the residual food recovered from the rainbow trout guts upon sacrifice. Based on this evidence, it appears that the formation and accumulation of octa- and heptaBDEs occurs at the gut wall. Work is currently in progress with intestinal and liver microsomes to assess the potential for debromination of BDE 209 and to compare the biotransformation of BDE 209 observed *in vivo*, to the *in vitro* experiments.

Reference List

1. Hites, R. A. (2004) *Environmental Science & Technology* 38, 945-956.

2. Stapleton, H. M., Dodder, N. G., Offenberg, J. H., Schnatz, M. M., Wise, S. A. (2005) *Environmental Science & Technology* 39, 925-931.

3. Hale, R. C., La Guardia, M.J., Harvey, E.P., Gaylor, M.O., Mainor, T.M., Duff, W.H., (2001) *Nature* 412, 140-141.

4. Soderstrom, G., Sellstrom, U., de Wit, C. A., Tysklind, M. (2004) *Environmental Science & Technology* 38, 127-132.

5. Stapleton, H. M., Alaee, M., Letcher, R. J. & Baker, J. E. (2004) *Environmental Science & Technology* 38, 112-119.

6. Kierkegaard, A., Balk, L., Tjarnlund, U., de Wit, C. A., Jansson, B. (1999) *Environmental Science & Technology* 33, 1612-1617.

7. Stapleton, H. M., Letcher, R. J., Baker, J. E. (2004) *Environmental Science & Technology* 38, 1054-1061.