Dietary exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE): Bioaccumulation Parameters and Biochemical Effects

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Introduction

Some chemical members of the family of bromine-based flame retardants are now ubiquitous in the environment. Brominated diphenyl ethers (BDEs), for example, have been detected globally in almost every environmental compartment and their detection has forced industry to phase out production of two formulations: penta and octa-mixtures. In response to this, the BFR industry has begun to market alternative bromine based compounds to replace these discontinued BDE formulations. One such compound, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), is thought to be replacing the octa-BDE mixture in some commercial applications. The recent detection of BTBPE in the atmosphere in the United States and also in biota samples from Lake Winnipeg (Canada) suggests that this compound is escaping from its intended material (Hoh and Hites (2005), Law *et al*. (2006a).

Our ongoing research efforts on BFRs have been to try and understand the impacts these chemicals may pose to the health of fish. In this study, juvenile rainbow trout (*Oncorhynchus mykiss*) held in the laboratory were exposed to an environmentally relevant concentration of BTBPE *via* the food. At different stages of the exposure experiment fish were sacrificed and muscle tissue and blood were analysed to assess the bioaccumulation potential and possible biochemical effects of BTBPE.

Materials and Methods.

Food preparation. Commercial fish food (Martin's Feed Mills, Elmira, ON, Canada) was placed in a pre-cleaned Hobart blender. Corn oil which was spiked with known amounts of BTBPE (x uL of 25 ng/uL solution in TMP) was transferred to the blender containing the food. After 20 minutes of gentle stirring, an aqueous gelatin binder (40g of gelatin in 1.5 L) was added. Stirring continued until a firm consistency was observed (~20 minutes). The resulting spiked food was air-dried for 40 minutes, extruded through a 4 mm diameter noodler, thoroughly dried at 10°C for 48 h and crushed into pellets. Control food was prepared in the same manner minus the test compounds. Concentrations of BTBPE were determined in control and spiked food using the same analytical techniques used to determine concentrations in the lake trout tissue described below.

Experiment. Juvenile rainbow trout (initial mean weights, 202 ± 7 g) were exposed to the spiked food for 49 d, followed by untreated food for 154 d. The daily feeding rate was equal to 1.0% of the mean weight of the fish, adjusted after each sampling period based on the mean weight of the sub-sample of fish that were sacrificed. 51 fish were used for each treatment and each treatment was held in separate 800 L fibreglass aquaria receiving 1.5 L UV and carbon dechlorinated Winnipeg city tap water/min (12°C) and

pH between 7.9 and 9.1. The dissolved oxygen was always at level of saturation. A 12 h light: 12 h dark photoperiod was maintained throughout the experiment. Four fish were sampled from each tank on days 0, 7, 14, 28 and 49 of the uptake period and on days 7, 14, 28, 56, 112, 203 d of the depuration period. Fish were always sampled 24 h after the previous feeding. Sampled fish were euthanized with an overdose (0.4 g/L) of pH buffered tricaine methanesulfonate anesthetic MS-222. After fin movement ceased (<3 min), 1 to 2 mLs of blood was obtained from the caudal vein of each fish. Blood samples were held on ice for < 1 hr and then centrifuged at 14 000 *g* for 5 minutes. Plasma was then transferred to a polyethylene microcentrifuge tubes, frozen immediately on dry-ice and then stored at -90°C protected from light until analyzed. Liver, bile, kidney, gastrointestinal (GI) tract and carcass (whole fish minus liver and GI tract) were then dissected from each fish. Liver and bile samples were frozen immediately on dry ice and later held at -90°C until analysis. Only tissue from the carcass was used for calculation of bioaccumulation parameters.

Biochemical analysis. Preparation of microsomes from liver tissue and analysis of T4 outer ring deiodinase enzyme activity in samples from Day 49 of the uptake phase were performed as described by Eales *et al.* 1999). Briefly, approximately 0.5 g of liver tissue was added to 10 volumes of ice cold 0.1 M Tris-HCl buffer (pH=7.6) that contained 0.1M NaCl. Livers were homogenized for 30 seconds with a Polytron homogenizer model PT 10/35 (Brinkman InstrumentsInc., Westbury, NY). The homogenate was centrifuged at 10,000 g for 20 minutes and the resulting supernatant was recovered and re-centrifuged at 105,000 g for 90 minutes. The pellet was recovered by pouring off the supernatant and the pellet was resuspended in 1 ml of 0.05 M Tris Buffer (pH=7.6), frozen in microcentrifuge tubes at -90ºC until utilized directly in the assay. Blanks were included in each analytical run to account for non-specific release of ^{125}I from T4. Free triiodothyronine (T3) and thyroxine (T4) were determined on day 49 of the uptake phase using 100 µL of plasma in a standard coated tube radioimmunoassay (ICN Pharmaceuticals, Orangeburg, NY).

Muscle tissue extraction and analysis. Details of the extraction and analysis of muscle tissue can be found in Law *et al*. (2006a).

Results and Discussion.

Bioaccumulation parameters. The uptake and clearance profile of BTBPE in lake trout is shown graphically in Figure 1. The uptake curve is linear (r^2=0.49, p< 0.02) and the uptake rate was calculated to be 0.23 nmole per day. It is clear that partition of BTBPE into the muscle tissue is still occurring even after the last day of exposure. After day 56, the depuration of BTBPE followed a first order decay process; a plot of ln of the concentration versus time was linear (r^2=0.52, p<0.008). Based on the slope of that line, a half-life ($t_{1/2}$) of 10.3 ± 3.1 days was determined. Compared to our other studies, the $t_{1/2}$ of BTBPE was much smaller than that of HBCD isomers (β: 157 and γ: 144 days) and congeners of BDEs (range: 26 to 173 days) (Law *et al.* 2006b, Tomy *et al*. 2005).

Biochemical effects and Histology. Examining potential thyroid axis disruption in animals exposed to BTBPE is warranted for several reasons. BTBPE is structurally similar to thyroid hormones and could be expected to potentially bind to thyroid receptors (Brown *et al*. 2004), as has been documented for brominated biphenyls with similar structures (Zhou *et al*. 2001). However, thyroid glandular structure appeared unaffected in fish exposed to the BTBPE concentrations in this study. Specifically, we found

similar epithelial cell heights and colloid storage (data not shown). Thorough examinations of the thyroid axis must consider not only glandular structure, but also the activity of deiodinase enzymes for converting inactive T4 to the more metabolically active T3, as well as concentrations of the active hormone in the blood (Eales *et al*. 1999). Neither blood plasma concentrations of T3 nor deiodinase enzyme activity were affected by BTBPE in this tudy. These results suggest that BTBPE is not as potent in terms of thyroid axis disruption as other brominated flame retardants. It may be that bromination of both phenolic rings at ortho positions results in BTBPE being less similar thyroid hormones and to other thyroid active polybrominated flame retardarants.

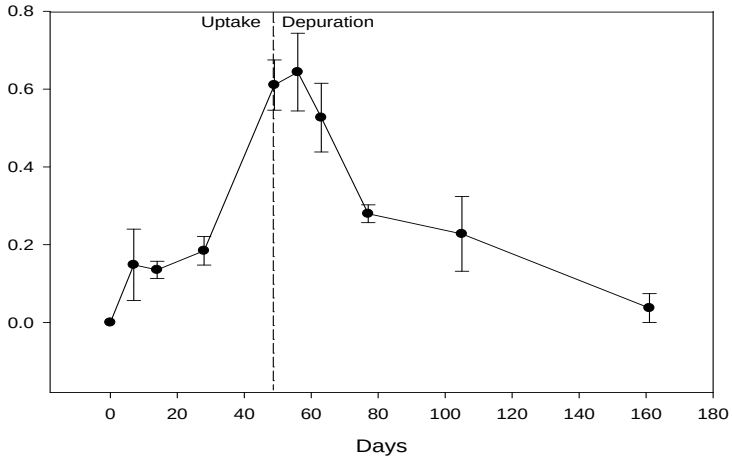

Figure 1. Uptake and depuration curves of BTBPE in muscle tissue of juvenile rainbow trout. Points are a lipid corrected mean of two fish (±1SE).

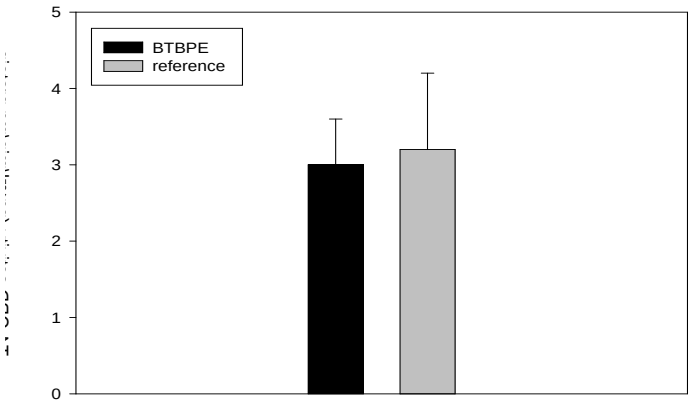

Figure 2. Heptaic deiodinase enzyme activity of the BTBPE exposed fish and the control fish at day 49 of the uptake phase.

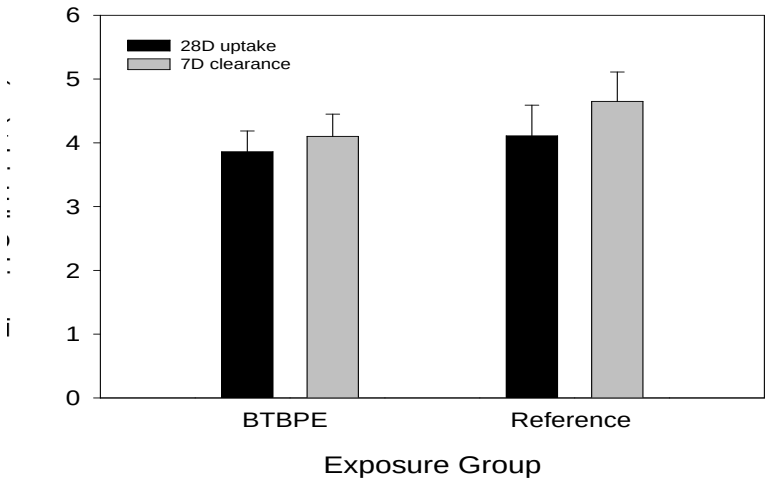

Figure 3. Histogram of thyroid cell height against BTBPE exposed fish and control fish at days 28 of the uptake and 7 of the clearance phase.

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