DOES ORAL EXPOSURE TO INDIVIDUAL HBCD DIASTEREOISOMERS ALTER THYROXINE (T4) METABOLISM IN JUVENILE RAINBOW TROUT?

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Introduction

Hexabromocyclodecane (HBCD) is the principle fire retardant in polystyrene foams that are used as insulation in the building industry and for upholstering furniture. The technical mixture consists of three diastereoisomers, α, β and γ. While the γ isomer is most often detected in abiotic compartments, there are differences in the composition of HBCD residues that are measured in biota. There is also little information on the relative potency of the diastereoisomers to induce biological effects in exposed organisms. Our earlier work suggested that fish exposed to HBCD exhibited an increase in the rate of turnover of thyroxine (T4), the precursor molecule to the active thyroid hormone triiodothyronine (T3)[1]. To examine this further, juvenile rainbow trout (*Oncorhynchus mykiss*) were held in the laboratory and fed diets containing environmentally relevant concentrations of the individual α, β and γ isomers. Thirty two (32) days after feeding began, twenty fish from each group were individually anesthetized, weighed and measured and then dosed by oral gavage with gelatin containing [^{125}I] T4. Measurements of the labeled T4 two days after gavage provided a means of determining tissue disposition and elimination rates of the hormone. In addition, by determining concentrations of native hormone in plasma and liver, ratios relative to the labeled dose in those tissues also provided a measure of turnover rates. Two days post gavage the β-HBCD diastereoisomer increased the deposition of [^{125}I] T4 in bile while pre-exposure to the γ-isomer reduced retention of [^{125}I] T4 in intestine and viscera. Monitoring of [^{125}I] T4 in tissues will continue at 4, 6 and 8 days post gavage to examine the potential for individual HBCD diastereoisomers to increase turnover rates of thyroid hormones and disrupt the thyroid axis.

Materials and Methods

Fish and Feeding

Juvenile rainbow trout (approximately 60 g) obtained from the Whiteshell Provincial Fish Hatchery (West Hawk Lake, Manitoba) were acclimated, 100 per tank, in 1600 liter fiberglass tanks receiving 2 L/min dechlorinated Winnipeg City tap (12±2ºC) water for >3 months prior to beginning the experimental feeding stages. During acclimation, fish were fed 5 pt Martin Mills trout chow at a ration of 1% of bodyweight 6 times per week. After the acclimation period, fish were redistributed so that each 1600 litre fiberglass tank held 40 fish. The fish weighed 175 ± 12 g at this point. Each group was fed 1% bodyweight 6 times per week of either the reference diet or diets enriched with α, β or γ-HBCD. The diets were formulated as described by Law et al. (2006). Briefly, 1.8 kg of powdered Martin Mills Silver Cup trout starter chow (Martin Mills, Elmira ON), with 40 ml corn oil (Sigma Chemical Co, St. Louis, MO) for the reference diet or corn oil containing α, β or γ-HBCD to attain a concentration of 5 ng/g in the final diet. The powdered starter food and corn oil was thoroughly mixed using a Hobart food grade mixer for 20 minutes after which 1.5 litres of DDW warmed to 37ºC containing 40 g of 60 bloom gelatin (Sigma Chemical Co.) was added. The entire mixture was allowed to mix for a further 20 minutes, followed by a drying period of 40-60 minutes. After drying, the food paste was extruded through a 5 pt die and the noodles were allowed to air dry at 25ºC for a period of 48 hours. Noodles were manually broken into pellets and the dry food was stored at -20ºC. Weighed portions of food were removed from storage daily, allowed to warm to room temperature (15 minutes) before being fed to the fish.

Oral Gavage

[^{125}I]T4 (ICN Biomedical, Boston MA) with a purity of >98% was suspended in 60 Bloom gelatin (Sigma Chem. Co) (0.075 g ml^{-1} DDW) warmed to 33ºC. Prior to dosing, each fish was anesthetized in MS-222 (0.1g l^{-1}) until fin movement ceased (<3 min) whereupon they were weighed, measured and administered the oral gavage dose of labeled T4. For each fish a 200 µl aliquot of gelatin containing 1 µCi of [^{125}I]T4 (specific activity 1200mCi/mg) was injected into the stomach using a 1 ml syringe, a 20 gauge blunt needle and 8.5 cm of #30

PTFE tubing. The tubing had been heat flared at the distal end to prevent tissue damage when the tube was guided down the esophagus and into the stomach. After the gavage dose was completed, each fish was allowed to recover (<3 min) in their original tanks (20 treated fish/tank).

Tissue Sampling and Analysis

Forty eight (48) hours after being orally dosed with [^{125}I]T4, each fish was individually anesthetized with 0.4 g/L MS-222 until fin movement ceased (<3min). After weighing and measuring, blood samples were obtained by caudal vein puncture. An aliquot of whole blood was used to determine radioactivity, and then plasma was obtained by centrifuging at 10,000Xg for 6 minutes. Plasma was frozen at -90ºC until it was analyzed. Liver, gall bladder containing bile, intestine and its contents and viscera (included stomach, adipose tissue and gonads), as well as four sub-samples of muscle were dissected. Radioactivity was determined in sub-samples taken from consistent locations from each tissue. Radioactivity in the entire lower jaw was also determined as a measure of the radioactivity in the thyroid, which in fish is a diffuse collection of follicles distributed throughout the lower jaw region[2]. Proportions of [^{125}I]T4 to dissociated [^{125}I] were determined in plasma by gel chromatography as described by Eales[2]. Briefly, 500 µl of plasma from each fish was loaded onto 0.25 g LH-20 Sephadex gel chromatography columns equilibrated in 0.1M HCl. Dissociated iodide was determined as the activity eluted from the columns with 5 ml of DDW. T4 associated activity was eluted with 5 mls of 0.1 M NH_4OH. All data are presented as Mean ± SEM and comparisons between groups were performed using ANOVA followed by Tukey's multiple comparison test with significance accepted at p=0.05.

Results and Discussion

We have previously demonstrated that circulating concentrations of free trriodothyronine (FT3) increase, while free thyroxine (FT4) decline in the circulation of juvenile rainbow trout exposed to individual HBCD diasteroisomers[1]. The thyroid axis itself appeared generally disrupted in these fish as evidenced by the accompanying thyroid gland hypertrophy and altered hormone metabolic enzymes. We had hypothesized that increased clearance rates of T4, specifically, might be responsible for the disruptions to the thyroid axis in HBCD-exposed fish. The primary route for inactivation and elimination of thyroid hormones is via glucuronate or sulphate conjugation with elimination in the bile and urine[3]. A greater percentage of the initial radioactive dose was recovered in the bile of fish fed the β-HBCD enriched food compared to fish fed the reference diet, suggesting that β-HBCD enhanced the elimination rate of [^{125}I]T4. Similar reductions of T4 have also been documented using other animal models[4].

While enhanced recovery of T4 or T4-metabolites in bile may indicate greater metabolism of T4, the relative retention of radioactivity in important thyroid hormone storage tissues may also provide some insight regarding turnover rates. Uptake of T4 from the gut, and distribution to the blood, is extremely low in teleosts[5] but even with relatively low assimilation rates, quantities of [^{125}I] T4 could be determined in several tissues. The muscle and liver are major storage sites for T4 and its metabolites, and the thyroid itself is a major site for iodide recovery. We also determined radioactivity in intestine and viscera because of the possibility that these tissues would retain a greater fraction of the unabsorbed oral dose or because of enhanced content of radioactivity arising from enterohepatic circulation of conjugated thyroid hormones.

Fish fed the γ-HBCD diet had lower proportions of the radioactive dose recovered in thyroid, intestine and viscera, possibly indicating that these fish assimilated less of the [^{125}I]T4 from the gavage injection (Table 1). Follow up studies determining the residual radiolabel quantities in each of the tissue at 4, 6 and 8 days post-gavage will provide critical data for evaluating the mechanisms by which HBCD alters T4 metabolism.

References

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Table 1: Percent recovery of initial radioactive T4 dose in tissues of juvenile rainbow trout fed formulated diets containing individual HBCD diastereoisomers. Data are presented as Mean ± SEM, n=4-5). Means marked with asterisks are significantly different from fish in the reference group, P<0.05.

		% Recovered Dose					
Group	n	Muscle	Thyroid	Intestine	Viscera	Liver	Blood
Reference	5	1.07 ± 0.17	0.80 ± 0.08	4.14 ± 1.77	1.16 ± 0.34	0.08 ± 0.03	1.11 ± 0.09
á- HBCD							

Figure 1: Percent recovery of initial radioactive T4 dose in bile of juvenile rainbow trout fed formulated diets containing individual HBCD diastereoisomers. Data are presented as Mean ± SEM, n=4-5. Bars marked with asterisks are significantly different from fish in the reference group, P<0.05.