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Characterizing the In Vitro Hepatic Biotransformation of the Flame Retardant BDE 99 by Common Carp

Pamela D. Noyes¹, Shannon M. Kelly¹, Carys L. Mitchelmore², and Heather M. Stapleton^{1,*}

¹ Duke University, Nicholas School of the Environment, Durham, NC, USA

² Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD, USA

Abstract

Polybrominated diphenyl ethers (PBDEs) are a class of flame retardant chemicals that are known to biomagnify in aquatic foodwebs. However, significant biotransformation of some congeners via reductive dehalogenation has been observed during *in vivo* and *in vitro* laboratory exposures, particularly in fish models. Little information is available on the enzyme systems responsible for catalyzing this metabolic pathway in fish. This study was undertaken to characterize the biotransformation of one primary BDE congener, 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), using *in vitro* techniques. Hepatic sub-cellular fractions were first prepared from individual adult common carp (*Cyprinus carpio*) to examine metabolism in both microsomal and cytosolic sub-cellular fractions. Debromination rates (i.e. BDE-99 biotransformation to BDE-47) were generally higher in the microsomal fraction than in the cytosolic fraction, and some intra-species variability was observed. Further experiments were conducted to determine the biotransformation kinetics and the influence of specific co-factors, inhibitors and competitive substrates on metabolism using pooled carp liver microsomes. The apparent K_m and V_{max} values were 19.4 μM and 1,120 pmoles hr^{-1} mg protein⁻¹, respectively. Iodoacetate (IaC) and the two thyroid hormones, reverse triiodothyronine (rT3) and thyroxine (T4), significantly inhibited the debromination of BDE-99 in microsomal sub-cellular fractions with IC_{50} values of 2.2 μM , 0.83 μM , and >1.0 μM , respectively. These results support our hypothesis that deiodinase enzymes may be catalyzing the metabolism of PBDEs in fish liver tissues. Further studies are needed to evaluate metabolic activity in other species and tissues that contain these enzymes.

Keywords

biotransformation; debromination; PBDEs; metabolism; and carp

1. INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are flame retardant chemicals applied to numerous types of polymers and resins to reduce their flammability. Because PBDEs are added and not chemically bound to these matrices, they can leach from products and accumulate in indoor

*Corresponding Author Address: Duke University, Nicholas School of the Environment, Durham, NC, 27708; Phone: (919) 613-8717; Fax: (919) 684-8741, heather.stapleton@duke.edu.

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and outdoor environments¹. PBDEs encompass as many as 209 different congeners, containing from one to ten bromine atoms substituted around a diphenyl ether backbone, similar to polychlorinated biphenyls (PCBs). PBDEs have different physical and chemical properties dependent largely on the number and substitution of bromine atoms. Generally, BDE congeners with six or fewer bromine atoms are more bioaccumulative and persistent, and potentially more toxic than the higher brominated congeners².

Several studies have investigated the bioaccumulation, metabolism and disposition of BDE congeners in different animal models³. Metabolism of BDE congeners in rodents appears to occur primarily through oxidative, cytochrome P450-mediated pathways (CYPs) that generate hydroxylated metabolites (e.g. OH-BDEs) similar to the metabolism of some PCB congeners⁴. In fish, however, studies that have examined the accumulation and metabolism of BDE congeners suggest that metabolism primarily occurs by a reductive dehalogenation pathway (Table 1) 2b, c⁵. No hydroxylated metabolites have been observed in PBDE- exposed fish. Recently, we confirmed with *in vivo* and *in vitro* testing that debromination of BDE congeners does occur through metabolic pathways in common carp (*Cyprinus carpio*)⁶. Recent studies have also found evidence to support reductive debromination of BDEs in rats⁷, lactating cows⁸, and European starlings⁹, suggesting that this metabolic pathway also occurs in mammals and birds, albeit likely as a minor pathway compared to oxidative routes.

While a growing body of evidence suggests that reductive debromination of BDE congeners is a major metabolic pathway in fish 2b, c^{5a}, 10, the involvement and role of specific enzyme systems are unknown. Previous work demonstrated that debromination of an environmentally relevant BDE congener, 2,2',4,4'-pentabromodiphenyl ether (BDE-99), occurs in carp and forms 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), which accumulates in carp tissues 2b. A follow-up study confirmed that this metabolic pathway occurs in carp liver and intestinal tissues, was not dependent upon the presence of the co-factor NADPH, and verified that debromination was not a function of gut microfauna 6b. However, no information is available on the sub-cellular location of PBDE debromination in fish hepatocytes (e.g. endoplasmic reticulum, cytosol, etc.). This is often a useful first step in identifying enzyme system(s) responsible for xenobiotic metabolism.

Major enzyme systems often responsible for the metabolism of xenobiotics include the CYPs that catalyze a majority of Phase I reactions and some isoforms of glutathione-S-transferases (GSTs), which are major Phase II conjugating enzymes¹¹. Studies have shown induction of CYP1A¹² while others have shown no response or inhibition of CYP1A in PBDE-exposed animals¹³. Moreover, induction of other CYP isoforms has been observed in rodents, including CYP2B¹⁴. Hepatic microsomal CYPs require NADPH and have broad substrate specificity allowing for oxidative and reductive metabolism of a spectrum of endogenous and xenobiotic agents. However, as stated previously, Benedict et al (2007) observed that carp debromination of BDE 99 was not dependent upon the presence of NADPH in the incubation buffer. Thus the probability that PBDE debromination is catalyzed by CYPs seems unlikely. BDE-glutathione metabolites have been observed in rodents^{3d},¹⁵ and avian species¹⁶, suggesting a possible role for GSTs, which are a supergene family that protect organisms from a variety of endogenous and xenobiotic agents. GSTs act by conjugating reduced glutathione to electrophilic centers on endogenous and exogenous compounds, and are also involved in transporting compounds through the cytoplasm¹¹,¹⁷. They can act by oxidative and reductive pathways and are widely distributed in cytosolic, microsomal, and mitochondrial loci with several different isoforms.

Because the metabolism of BDE 99 is not well characterized, additional approaches are needed to elucidate the enzyme(s) involved in PBDE debromination. Alternate approaches include identifying the sub-cellular locations of metabolism, withholding specific enzyme cofactors,

and adding enzyme inhibitors and substrate competitors. This study was undertaken to further characterize the metabolism of BDE-99 in common carp liver tissue using *in vitro* techniques to help differentiate the roles of suspected enzyme systems, including the GSTs and deiodinases (DIs). Our previous research has implicated DIs in catalyzing debromination of BDE congeners in carp and rainbow trout 2b, 6. The three known isoforms of DI (Type 1, 2, and 3) are typically associated with the microsomal fraction and regulate intracellular levels of thyroid hormones, such as thyroxine (T4) and triiodothyronine (T3), by catalyzing deiodination 18. This process parallels the metabolism of BDE-99 (structurally similar to T4; Figure 1) to BDE-47 observed by Stapleton et al. (2004b) whereby a bromine atom is selectively removed (i.e. debromination) from the meta-position of the diphenyl ring (Figure 1).

The specific objectives of this study were: to compare the metabolism of BDE-99 in carp liver microsomal and cytosolic sub-cellular fractions; to determine the maximum velocity ($V_{\max}$) and the Michaelis constant (K_m) to describe the biotransformation kinetics of BDE-99 metabolism in carp; and to characterize enzyme(s) modulating metabolism by comparing the influence of specific co-factors, enzyme inhibitors, and putative competitive substrates (e.g. thyroid hormones) on the biotransformation rate of BDE-99.

2. MATERIALS AND METHODS

2.1. Animals

Common Carp (*Cyprinus carpio*) were purchased as juveniles from Hunting Creek Fisheries (Thurmont, MD) and reared at the Chesapeake Biological Laboratory in Solomons, MD for over a year prior to this study. Using approved animal care guidelines, carp were reared in tanks with flow-through ambient water (18–22 °C) and a 12-hour photoperiod, and were fed 3–5% of their body weight daily with standard pelleted Koi food. Seven adult male carp were randomly selected for this study. The fork length of the animals ranged from 243–297 mm and their body masses ranged from 445–673 g. Fish were sacrificed and their livers dissected, washed in a phosphate buffer, and immediately frozen in liquid nitrogen for transport (on dry ice) to Duke University.

2.2. Materials

Internal and surrogate standards (^{13}C labeled 2,2',3,4,5,5'-chlorinated diphenyl ether (^{13}C -CDE-141) and 4'-fluoro-2,3',4,6-tetrabromodiphenyl ether (F-BDE-69)) used in this study were purchased from Chiron (Trondheim, Norway). PBDE quantification standards were purchased from Accustandard (New Haven, CT). All solvents used throughout this study were High Performance Liquid Chromatography (HPLC) grade.

2.3. Hepatocyte sub-cellular fraction preparations

Hepatocyte sub-cellular fractions were prepared as previously described¹⁹. In one experiment, described below, DTT was excluded from the washing buffer to evaluate its role in BDE-99 metabolism. The amount of re-suspension buffer added to the microsomal pellets was equivalent to 1 mL/g of liver tissue. As quality control to verify the viability of the sub-cellular fractions (i.e., confirmation that the sub-cellular fractions are active), an assay was conducted for measuring GST enzyme activity toward 1-chloro-2,4-dinitrobenzene (CDNB). As various isoforms of GST exist in both microsomal and cytosolic fractions, CDNB is a relatively nonspecific reference substrate for most GST isoforms. The spectrophotometric method of Habig and Jakoby (1981) was employed using a 96-well microplate reader, performed at 25 °C in a reaction buffer containing 1 mM of reduced glutathione (GSH) (pH 6.5). Following addition of 1 mM CDNB, absorbance was measured at 340 nm for 5 min. GST activity toward CDNB ranged from 140–236 nmol min⁻¹ mg protein⁻¹ in microsomes, and 148–448 nmol

$\text{min}^{-1} \text{mg protein}^{-1}$ in cytosol. These values are all within normal ranges for carp, suggesting that the isolated sub-cellular fractions were viable ²⁰.

2.4. BDE-99 incubations with sub-cellular fractions

All incubations were conducted in glass test tubes and contained either 900 μL or 950 μL of incubation buffer, 100 μL or 50 μL of the appropriate sub-cellular fraction, and 354 pmoles of BDE-99 for a total volume of 1 mL/incubation. The enzyme kinetic experiment used varying substrate concentrations. The buffer used for all the incubations consisted of 0.1 M potassium phosphate (K_2HPO_4), 0.1 M sodium phosphate (NaH_2PO_4), and 100 μM NADPH (pH 7.4). In addition, all incubation buffers contained 10 mM of dithiothreitol (DTT), with the exception of one experiment investigating DTTs role in BDE-99 debromination. All incubations were conducted for 60 minutes at 25 °C in a water bath with continuous shaking at 140 rpm. Non-enzymatic controls used in the experiments included inactivated sub-cellular fractions (i.e. immersed in boiling water for 10 min) treated with BDE-99 and incubated alongside the active sub-cellular fractions. At the conclusion of the incubation period, 1 mL of ice cold methanol was added to halt the reactions. Samples were stored at 4 °C until extraction for analysis. Protein concentrations of all the carp hepatocyte fractions ranged from 10.86–21.54 mg/mL and were determined with a bicinchoninic acid assay (BCA) kit (Pierce, Rockford, IL). Concentrations of metabolites formed were normalized to time and protein concentration.

Three sets of experiments were undertaken. In the first experiment, microsomal and cytosolic fractions isolated from the liver tissue of 7 juvenile carp were incubated with BDE-99 to evaluate the sub-cellular site of metabolic debromination and intra-species variability. For each specimen, 50 μL of microsomes or cytosol were incubated in 950 μL of incubation buffer solution and 354 pmoles of BDE-99. This BDE-99 treatment was selected based on incubation conditions from our previous *in vitro* work in carp hepatocytes and to minimize exposure to the acetone diluents used at 0.5% per incubation ^{6a}. In the second set of experiments, pooled carp hepatic microsomes (from the specimens in the first experiment) were exposed to a range of substrate conditions in triplicate to determine the V_{max} and K_m values of BDE-99 biotransformation to BDE-47. Substrate concentrations ranged from 1–250 μM of BDE-99 (based on solubility and amount of BDE-99 available). In separate tests, BDE-99 incubations at the 50 μM treatment were extended to 16 and 24 hours in triplicate to evaluate catalytic debromination of BDE-99 over time.

In the final experiments, hepatic microsomes from pooled carp livers were incubated with and without enzyme inhibitors, substrate competitors, and DI cofactors. Each incubation contained 900 μL of incubation buffer and 100 μL of hepatic microsome incubated with 354 pmoles of BDE-99. The enzyme inhibitors used in the challenge were 10 mM of propyl-thiouracil (PTU) and 0.1–100 mM of iodoacetate (IaC). The substrate competitors used were 0.001–2.0 μM of reverse triiodothyronine (rT3) and 0.0001–1.0 μM of thyroxine (T4). The *in vitro* DI cofactor used was 10 mM of DTT. Using the CDNB assay described in Section 2.3, GST activity was measured in buffers containing rT3 in concentrations ranging from 0–2.0 μM .

2.5 Sample extraction and analysis

All samples were spiked with 50 ng of a recovery standard (F-BDE-69) and subjected to a liquid-liquid extraction procedure as described previously ^{6b}. Hexane was added to glass test tubes containing samples, and centrifuged to separate out the organic fractions. The organic layer was transferred to a glass test tube and the extraction was repeated twice more. The organic extractions were then treated and centrifuged with concentrated sulfuric acid to remove biogenic material. The final combined extract was concentrated to a volume of 0.5 mL using a rapid evaporation system with ultra-high grade nitrogen gas and 50 ng of an internal recovery standard (^{13}C -CDE-141) was added in preparation for analysis.

All samples were analyzed using gas chromatography mass spectrometry (Agilent models 6890N and 5975) operated in negative chemical ionization mode (GC/ECNI-MS). Extracts were analyzed for a suite of 12 BDE congeners ranging from tri- to penta-BDE. The operating conditions for the GC/MS were the same as those described previously²¹. A 0.25 mm (I.D.) × 15 m fused silica capillary column coated with 5% phenyl methylpolysiloxane (0.25 µm film thickness) was used for the separation of BDE congeners. Pressurized temperature vaporization (PTV) injection was employed in the GC. The tri- through penta-BDE congeners were quantified by monitoring bromide ions m/z 79 and 81 in Selective Ion Monitoring (SIM) mode.

2.6 Quality Assurance

Recovery of F-BDE-160 averaged $80.5 \pm 20\%$. BDE-99 and BDE-47 were detected in laboratory blanks; however, levels were $<1\%$ of measured values making blank correction of the samples immaterial since these negligible amounts did not affect the measured values reported under our method. Limits of detection (LOD) were defined as three times the standard deviation of laboratory blanks. For congeners not detected in the blanks, the LOD was set at the lab limit of quantification (LOQ).

2.7 Data Analysis

The rate of metabolite (e.g., BDE-47) formation was determined in all treatments and the data are presented as units of pmoles of BDE-47 produced $\text{hour}^{-1} \text{mg protein}^{-1}$. Data were analyzed using StatView 5.0 (SAS, Inc., Cary, NC) and Microsoft Excel (2007) with statistical significance defined at the $p < 0.05$ level. Differences in the formation rate of BDE-47 among individual carp were evaluated using a single factor ANOVA and Fisher's Protected Least Significant Difference (PLSD) post hoc test for multiple pair-wise comparisons. We also used t-tests to test for significant differences among active and inactive fractions, including those fractions incubated with inhibitors, cofactors, and competitive substrates. $V_{\max}$ and K_m values in pooled liver microsomal fractions were calculated using SigmaPlot 9.0 (Point Richmond, CA) with non-linear and linear regression analysis and ligand binding models. Additional confirmatory line-fitting analysis was undertaken using Microsoft Excel (2007).

3. RESULTS

3.1 Sub-cellular biotransformation among individual carp specimens

Significant ($p < 0.05$) debromination of BDE-99 was observed among all the microsomal and cytosolic fractions prepared from the individual carp (Figure 2). No BDE-99 debromination was observed in heat inactivated incubations. Biotransformation rates in the microsomal fractions ranged from 39.6 ± 6.50 to 167 ± 17.2 pmoles BDE-47 $\text{hour}^{-1} \text{mg protein}^{-1}$ (mean $\pm$ SEM; $n=3$). In the cytosolic fraction, biotransformation rates ranged from 9.34 ± 0.58 to 89.3 ± 21.5 pmoles BDE-47 $\text{hour}^{-1} \text{mg protein}^{-1}$ (mean $\pm$ SEM; $n=3$). The rate of BDE-47 formation was significantly higher ($p < 0.05$) in the microsomal fraction than in the cytosolic fraction of fish 1, 3, 5, 7, whereas no differences were detected in the other three specimens. The biotransformation rate of BDE-99 was 15X higher in the microsomal fraction relative to the cytosolic fraction in specimen 1, and was 1.5X–3.5X higher in the other specimens. Significant ($p < 0.05$) inter-individual variability in debromination rates was observed among some of the carp specimens (e.g., fish 1 and 7), although there was also no difference in BDE-47 formation among some individuals (e.g., fish 2 and 4).

3.2 Enzyme Kinetics

The apparent $V_{\max}$ and K_m values for BDE-47 formation were at least 1,120 pmoles BDE-47 $\text{hour}^{-1} \text{mg protein}^{-1}$ and 19.4 µM BDE-99, respectively, in pooled carp microsomes incubated with BDE-99 at concentrations ranging from 1–250 µM (the highest concentration we could

test given the material available). As seen in Figure 3, the observed biotransformation rates increased with BDE-99 concentrations following first-order kinetics, which was potentially followed by zero-order kinetics upon enzyme saturation of the substrate. However, it is possible that we did not achieve full substrate saturation as the linear and nonlinear regression analyses of the data were equally robust. It is also notable that other tetraBDE congeners, BDE-66 (2,3',4,4'-tetraBDE) and BDE-49 (2,2',4,5'-tetraBDE), as well as small amounts BDE-28/-33 were measured in incubations containing >125 μM of BDE-99 (Figure 3). In the 250- μM treatment group, approximately 23% and 6% of the debromination products by mass were BDE-66 and BDE-49, respectively; while approximately 4% was either BDE-28 or BDE-33 (these congeners co-elute using our GC/ECNI-MS method). In the 125- μM treatment group, approximately 14% and 5% of the metabolites measured were BDE-66 and BDE-49, respectively.

These results have been blank corrected to account for small amounts (<1%) of these congeners observed in our dosing solutions; however, it is important to note that these congeners were not observed in our heat-inactivated controls, suggesting they were formed via metabolism. At lower substrate concentrations (1–50 μM of BDE-99), BDE-47 dominated nearly all the metabolite profile with only negligible amounts of these other congeners detected. Sustained velocity of BDE-47 formation was also measured at approximately 600 $\text{pmoles hr}^{-1} \text{ mg protein}^{-1}$ in the 50- μM treatment group subjected to extended incubations for up to 24 hours (maximum time evaluated).

3.3. Effects of Inhibitors, Substrate Competitors, and Cofactors

IaC had a significant ($p < 0.05$) effect on the biotransformation rate of BDE-99 in pooled liver microsomes (Figure 4). The concentration that inhibited the debromination by 50% (half-maximal inhibitory concentration or IC_{50}) was approximately 2 mM IaC. No inhibition of BDE-47 was observed in pooled hepatic microsomal fractions challenged with 10 mM PTU (data not shown). However, BDE-99 metabolism dropped significantly ($p < 0.05$) when DTT was removed from the incubation buffer and only retained in the homogenization buffer of pooled hepatic microsomes (3 replicates, 2 carp). Specifically, with DTT in the homogenization and incubation buffers of pooled microsomal fractions, the rate of BDE-47 formation was $120 \pm 7 \text{ pmoles hr}^{-1} \text{ mg protein}^{-1}$, whereas removing DTT from the incubation buffer but retaining it in the homogenization buffer reduced the BDE-47 formation rate to $5.3 \pm 1.1 \text{ pmoles hr}^{-1} \text{ mg protein}^{-1}$.

As seen in Figure 5, both T4 and rT3 substrate competitors had significant ($p < 0.05$) effects on the BDE-99 biotransformation rates when added to the incubation buffer. Specifically, incubations with 200 nM of rT3 reduced the metabolite formation rate, but only incubations with 1.0 and 2.0 μM rT3 were significant ($p < 0.05$). In the T4 treatment, only the highest level tested, 1.0 μM , had a significant ($p < 0.05$) effect on the metabolite formation rate. Based on these assays, the IC_{50} value calculated for rT3 was 0.83 μM , whereas the value for T4 was greater than 1.0 μM . Finally, microsomal GST activity (as measured by the CDNB assay) was found to be relatively consistent among all rT3 treatments tested in pooled carp liver microsomal fractions and no statistically significant ($p > 0.05$) differences were observed (Table 1).

4. DISCUSSION

4.1 Sub-cellular metabolism

Our data suggest the enzymes catalyzing the metabolism are more prevalent in the microsomal fraction. Xenobiotic metabolizing enzyme systems known to be present in microsomal fractions that may be capable of catalyzing PBDE metabolism include the CYP enzymes,

uridinediphosphate-glucuronosyltransferases (UGTs), and a few isoforms of GSTs. CYP enzymes have been known to catalyze reductive reactions, but these typically occur under low oxygen conditions¹¹. Furthermore, previous work indicated that BDE-99 metabolism in carp liver microsomal fractions was not dependent on NADPH in the incubation buffer 6b. As such, the likelihood that CYPs are involved seems unlikely.

The phase II metabolizing enzymes, UGTs and GSTs, have also been proposed to play a role in PBDE biotransformation via conjugation processes 12a²². Since our incubation buffer does not contain the co-factor, uridinediphosphate glucuronic acid, it seems unlikely that UGTs are modulating BDE-99 metabolism in carp. GSTs may catalyze the debromination of BDE-99 as there are microsomal (MAPEGs) and cytosolic isoforms, as well as small amounts of GSH present in our sub-cellular fractions. As described in the methods section, a common assay used to measure GST activity in sub-cellular fractions involves a nucleophilic substitution reaction whereby GST catalyzes the conjugation of GSH to CDNB, resulting in a glutathione-2,4-dinitrobenzene product. Thus, GSTs appear to be capable of dehalogenation reactions. However, Browne et al (2009) observed no change in BDE-47 debromination rates in dialyzed carp cytosol (i.e. cytosol containing no endogenous GSH) when exogenous GSH was excluded or included in their incubations. Thus, these data suggest that cytosolic GSTs do not appear to be involved in the debromination of BDE-99 debromination in carp.

Other enzymes that we have previously hypothesized as being involved in BDE-99 metabolism are the DIs. Studies have shown²³²³²³²³²³²³¹⁸¹⁸ that Type 1 DI has an NH₂-terminus located in the endoplasmic reticulum while the catalytically active COOH-terminus is in the cytosol (Baqui et al., 2000; Toyoda et al., 1995). *In vitro* studies have also shown that Type 1 DI is localized to the plasma membrane, suggesting that this DI isoform is synthesized in the ER and then transported to the plasma membrane (Leonard et al., 1991; Toyoda et al., 1995). In other tissues, a study using cerebral cortex tissue from hypothyroid rats observed limited deiodinase activity in both microsomal and cytosolic fractions²⁴, as did a study on human thyroid tissues²⁵ suggesting some DI activity in the cytosol. In addition, DIs may localize to the cytosol, possibly in a transient fashion as they are transported to plasma membranes, allowing for limited metabolic activity in this fraction. Based on this line of evidence, the metabolic activity we observed in the cytosolic fraction may be attributable to DIs having a relatively weak association with the ER or being transiently localized in the cytosol. Since the DI enzymes in carp have not been fully characterized these hypotheses require more study.

4.2 Enzyme Kinetics

It is difficult to compare the enzyme kinetic rates we observed in carp hepatic microsomes incubated with BDE-99 to literature values as enzyme kinetics can vary quite significantly within and among species, different enzyme systems, and between and across endogenous and xenobiotic substrates. While we cannot be certain that substrate saturation was achieved, the apparent K_m values (19.4 μM BDE-99) we measured are generally consistent with studies exploring the hepatic enzyme kinetics in carp and additional teleost hepatic microsomes incubated with other xenobiotic metabolizing enzymes²⁶. Additional examination of the enzymatic kinetics of BDE-99 debromination in other species is needed to evaluate the magnitude of the values we measured.

It is interesting that we observed a dose-response formation of two other tetraBDE congeners of note, BDE-66 and BDE-49 (Figure 3). While the congener pattern was dominated by BDE-47, akin to T4 to T3 conversions via DIs, at higher substrate concentrations BDE-66 and BDE-49 constituted appreciable percentages of the metabolite profile. The formation of BDE-49 has been observed in other teleosts, notably salmon^{10a}. Figure 6 proposes a metabolic debromination pathway for BDE-99 in carp at these higher substrate concentrations. While the majority of BDE-99 metabolism in carp is dominated by the meta-substitution of bromine to

produce BDE-47, at higher substrate concentrations, we observe the potential for cleavage of ortho- and para-substituted bromines to produce BDE-66 and BDE-49, respectively. Finally, the significant ($p < 0.05$) albeit small amounts of the triBDE congeners BDE-28 or BDE-33 (these congeners co-elute with our analytical method) formed may be suggestive of further meta-, ortho-, and/or para- cleavage of bromine from diphenyl ether. The underlying enzymatic pathways driving this biotransformation at elevated substrate concentrations require further characterization, and may point to the involvement of other enzymatic systems, in addition to the DIs.

The enzyme systems catalyzing debromination of BDE-99 in carp liver microsomes also sustained high *in vitro* activity (approximately 600 pmoles hr⁻¹ mg protein⁻¹) for an extended period lasting at least 24 hours. This observation supports that the enzyme systems catalyzing BDE-99 metabolism are capable of maintaining activity for an extended period in carp, and further discounts the role of some enzymes, notably CYPs. For example, CYP1A activity in human hepatic microsomes maintained at 25 °C was observed to cease after 6 hours²⁷. While this result was seen in human microsomes, a number of other studies examining PBDE metabolism in fish have shown that the CYPs are probably not catalyzing PBDE debromination^{6b, 10a, 28}.

4.3 Influence of co-factors, inhibitors, and substrate competitors

Because BDE-99 is metabolized in carp through a reductive dehalogenation pathway, and since our previous work has implicated involvement of DIs, we examined the effects of enzyme inhibitors, substrate competitors, and DTT *in vitro* co-factor on BDE-99 metabolism. Propylthiouracil (PTU) is a specific inhibitor of Type 1 DI that works well in mammalian liver tissues and is often used to differentiate the role of Type 1 and Type 2 DIs²⁹. While we observed no effects in our carp hepatic microsomes challenged with PTU (i.e., no inhibition of BDE-47 formation), studies have demonstrated that Type 1 DI in teleosts can be resistant to PTU³⁰. For example, 1 mM of PTU has been observed to cause only weak inhibition of Type 1 DI in tilapia 30b, trout 30a, and killifish 30d.

IaC is also an inhibitor of DI activity and we found significant ($p < 0.05$) inhibition of BDE-99 biotransformation in the presence of IaC. This compound has been shown to inhibit Type 1 DI more effectively than Type 2 or 3 DIs³¹. Our results with IaC demonstrate a possible role for Type 1 DI in BDE-99 metabolism. However, IaC inhibitory action occurs via the alkylation of cysteine residues at enzyme active centers. It is therefore not a specific DI inhibitor but probably a promiscuous inhibitor of many enzyme systems that contain cysteine residues. For example, IaC would likely inhibit GST activity by alkylating cysteine residues at GSH binding sites³². Nonetheless, previous studies have measured a dose dependent inhibition of rT3-ORD, T4-ORD, and T3-IRD among several fish species exposed to IaC, with IC₅₀ values in liver tissues ranging from 0.6 mM to >>1 mM³³. Thus the IC₅₀ value we observed of approximately 2 mM is in the range reported for inhibition of DI activity.

DTT is a potent reducing agent and is used to reduce disulfide bonds in proteins (e.g. DIs), and specifically to prevent disulfide bonds from forming between active sites in selenocysteine residues. DTT is generally regarded as an essential thiol co-factor for investigating DI activity in *in vitro* assay systems^{30a, 34}. Specifically, studies examining DI activity in fish hepatic microsomes have found that DTT is required to maintain DI activity and that increasing the amount of DTT can often increase the level of DI activity^{30a, 34}. BDE-99 biotransformation in carp hepatic and intestinal microsomal fractions was found to not be dependent on the presence of NADPH, suggesting that CYP enzymes were not mediating the reaction^{6b}. Therefore, in the present study we investigated the effect of DTT in the incubation buffer of pooled carp hepatic microsomes. We were only able to evaluate this relationship with livers from two carp and so further study is needed to fully evaluate the influence of DTT on BDE-99

metabolism. Nonetheless, our observations of enhanced activity in incubation buffers containing DTT and depressed activity in buffers without DTT suggest that this co-factor is important to BDE-99 catalytic activity in carp. This provides further evidence suggesting the involvement of DI enzymes in BDE biotransformation in fish.

To further investigate the role of DIs in BDE-99 biotransformation, several competitive substrate incubations were conducted using rT3 and T4. In a previous study, 0.77 μM of rT3 was found to significantly inhibit the BDE-99 debromination reaction by almost 50% in both intestinal and hepatic carp liver microsomal fractions^{6b}. Because the DI-catalyzed K_m value for T4 is typically low (0.5–1.0 nM) relative to rT3 (8–180 nM) in fish liver tissues^{30d, 34}, we selected different test concentrations for T4 and rT3. The T4 ranges were selected to bracket known K_m values for DI activity, and thus ranged from 0.1 nM to 1.0 μM , whereas the range of rT3 levels used ranged from 20 nM to 2.0 μM . We found that rT3 and T4 appeared to act as competitive substrates and/or inhibit the catalytic activity of the mediating biotransformation enzyme, with rT3 acting as a more potent inhibitor ($\text{IC}_{50} = 0.83 \mu\text{M}$) than T4 ($\text{IC}_{50} > 1$).

Type 1 and Type 2 DIs are known to be present and active in fish hepatocytes³³. Type 1 DI catalyzes both outer and inner ring deiodination of T4 and has a substrate preference for rT3. Type 2 DI catalyzes only outer ring deiodination of T4 so has a substrate preference for T4²⁹. Our finding that rT3 and T4 both inhibited BDE-99 biotransformation points to the potential activity of DI enzymes in catalyzing this activity. This is supported by findings that IaC inhibited the biotransformation with DTT acting as a potentially necessary cofactor. Moreover, rT3 appeared to be a stronger inhibitor of BDE-99 biotransformation than T4, suggesting a potentially more prominent role for Type 1 DIs in catalyzing this reaction.

With regard to the GSTs, research by Browne et al. (2009) suggests that cytosolic GSTs are not metabolizing BDE-99 in carp. For microsomal GSTs, we might expect that if rT3 inhibits MAPEG activity, this inhibition may be responsible for the observed decrease in BDE-99 metabolism observed with increasing rT3. However, we observed no decrease in MAPEG activity from co-incubation with rT3 (Table 2) so this metabolic pathway in fish microsomes seems unlikely.

5. CONCLUSIONS

This study has confirmed that carp hepatocytes can effectively debrominate BDE-99 via a reductive pathway and that the enzymes catalyzing this reaction are likely associated with the endoplasmic reticulum (e.g. microsomal fraction). However, cytosolic debromination was also observed, making it difficult to fully pinpoint the sub-cellular site of biotransformation. This outcome is distinguished from results observed in human liver hepatocytes whereby no reductive debromination was observed^{12a}. Debromination of BDE-99 was significantly inhibited by the presence of iodoacetate (IaC) and thyroid hormones (rT3 and T4). Inhibition by IaC suggests the involvement of DIs and GSTs, which both contain cysteine residues at their active sites. However, our results showing no change in MAPEG activity upon challenge with rT3 seems to discount their involvement in BDE-99 metabolism, while results by Browne et al. (2009) suggests GSTs appear to not be involved in cytosolic debromination. The inhibition by rT3 and T4 seems to implicate a greater role for DIs in catalyzing the reaction. Moreover, stronger inhibition by rT3 as opposed to T4 would also appear to implicate a role for Type 1 DIs in carp hepatocytes. Further studies are needed to confirm the influence of PBDEs on hepatic DI activity in carp tissues and other teleosts to conclusively identify the specific enzyme systems involved in PBDE debromination. In addition, it would be informative to examine the effects of the PBDEs on thyroid hormone metabolism.

References

1. (a) Hale RC, La Guardia MJ, Harvey E, Gaylor MO, Mainor TM. Brominated flame retardant concentrations and trends in abiotic media. *Chemosphere* 2006;64(2):181–186. [PubMed: 16434082] (b) de Wit CA, Alaee M, Muir DCG. Levels and trends of brominated flame retardants in the Arctic. *Chemosphere* 2006;64(2):209–233. [PubMed: 16458344] (c) Alaee M, Arias P, Sjodin A, Bergman A. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environment Int* 2003;29(6):683–689.
2. (a) Burreau S, Axelman J, Broman D, Jakobsson E. Dietary uptake in pike (*Esox lucius*) of some polychlorinated biphenyls, polychlorinated naphthalenes and polybrominated diphenyl ethers administered in natural diet. *Environ Toxicol Chem* 1997;16(12):2508–2513. (b) Stapleton HM, Letcher RJ, Li J, Baker JE. Dietary accumulation and metabolism of polybrominated diphenyl ethers by juvenile carp (*Cyprinus carpio*). *Environ Toxicol Chem* 2004;23(8):1939–1946. [PubMed: 15352483] (c) Tomy GT, Palace VP, Halldorson T, Braekevelt E, Danell R, Wautier K, Evans B, Brinkworth L, Fisk AT. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus namaycush*). *Environ Sci Technol* 2004;38(5):1496–1504. [PubMed: 15046352] (d) Birnbaum LS, Staskal DF. Brominated flame retardants: Cause for concern? *Environ Health Perspect* 2004;112(1):9–17. [PubMed: 14698924]
3. (a) Staskal DF, Hakk H, Bauer D, Diliberto JJ, Birnbaum LS. Toxicokinetics of polybrominated diphenyl ether congeners 47, 99, 100, and 153 in mice. *Toxicol Sci* 2006;94(1):28–37. [PubMed: 16936226] (b) Morck A, Hakk H, Orn U, Wehler EK. Decabromodiphenyl ether in the rat: Absorption, distribution, metabolism, and excretion. *Drug Metab Dispos* 2003;31(7):900–907. [PubMed: 12814967] (c) Burreau S, Broman D, Orn U. Tissue distribution of 2,2',4,4'-tetrabromo[¹⁴C]diphenyl ether ([¹⁴C]-PBDE 47) in pike (*Esox lucius*) after dietary exposure - a time series study using whole body autoradiography. *Chemosphere* 2000;40(9–11):977–985. [PubMed: 10739035] (d) Hakk H, Larsen G, Klasson-Wehler E. Tissue disposition, excretion and metabolism of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) in the male Sprague-Dawley rat. *Xenobiotica* 2002;32(5):369–382. [PubMed: 12065060]
4. (a) Marsh G, Athanasiadou M, Athanassiadis I, Sandholm A. Identification of hydroxylated metabolites in 2,2',4,4'-tetrabromodiphenyl ether exposed rats. *Chemosphere* 2006;63(4):690–697. [PubMed: 16213553] (b) Qiu X, Mercado-Feliciano M, Bigsby RM, Hites RA. Measurement of Polybrominated Diphenyl Ethers and Metabolites in Mouse Plasma after Exposure to a Commercial Pentabromodiphenyl Ether Mixture. *Environ Health Perspect* 2007;115(7):1052–1058. [PubMed: 17637922] (c) Letcher, RJ.; Klaassen-Wehler, E.; Bergman, A. Methyl Sulfone and Hydroxylated Metabolites of Polychlorinated Biphenyls. In: Paasivirta, J., editor. *The Handbook of Environmental Chemistry*. Springer-Verlag; Berlin: 2001. p. 315-359.
5. (a) Kierkegaard A, Balk L, Tjarnlund U, De Wit CA, Jansson B. Dietary uptake and biological effects of decabromodiphenyl ether in rainbow trout (*Oncorhynchus mykiss*). *Environ Sci Technol* 1999;33(10):1612–1617. (b) Stapleton HM, Letcher RJ, Baker JE. Debromination of polybrominated diphenyl ether congeners BDE 99 and BDE 183 in the intestinal tract of the common carp (*Cyprinus carpio*). *Environ Sci Technol* 2004;38(4):1054–1061. [PubMed: 14998018]
6. (a) Stapleton HM, Brazil B, Holbrook RD, Mitchelmore CL, Benedict R, Konstantinov A, Potter D. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. *Environ Sci Technol* 2006;40(15):4653–4658. [PubMed: 16913120] (b) Benedict RT, Stapleton HM, Letcher RJ, Mitchelmore CL. Debromination of polybrominated diphenyl ether-99 (BDE-99) in carp (*Cyprinus carpio*) microflora and microsomes. *Chemosphere* 2007;69(6):987–993. [PubMed: 17640709]
7. Huwe JK, Smith DJ. Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male Sprague-Dawley rats following dietary exposure. *Environ Sci Technol* 2007;41(7):2371–2377. [PubMed: 17438789]
8. Kierkegaard A, Asplund L, de Wit CA, McLachlan MS, Thomas GO, Sweetman AJ, Jones KC. Fate of higher brominated PBDEs in lactating cows. *Environ Sci Technol* 2007;41(2):417–423. [PubMed: 17310701]

9. Van den Steen E, Covaci A, Jaspers VLB, Dauwe T, Voorspoels S, Eens M, Pinxten R. Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (*Sturnus vulgaris*). *Environ Pollut* 2007;148(2):648–653. [PubMed: 17239511]
10. (a) Browne EP, Stapleton HM, Kelly SM, Tilton SC, Gallagher EP. In vitro hepatic metabolism of 2,2',4,4',5-pentabromodiphenyl ether (BDE 99) in Chinook Salmon (*Onchorhynchus tshawytscha*). *Aquat Toxicol* 2009;92(4):281–287. [PubMed: 19346012] (b) Stapleton HM, Alaei M, Letcher RJ, Baker JE. Debromination of the flame retardant decabromodiphenyl ether by juvenile carp (*Cyprinus carpio*) following dietary exposure. *Environmental Science & Technology* 2004;38(1):112–119. [PubMed: 14740725]
11. Di Giulio, RT.; Benson, WH.; Sanderson, JT.; Van Veld, PA. Biochemical mechanisms: Metabolism, Adaptation, and Toxicity. In: Rand, GM., editor. *Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment*. CRC Press; Boca Raton: 1995. p. 523-561.
12. (a) Stapleton HM, Kelly SM, Pei R, Letcher RJ, Gunsch C. Metabolism of Polybrominated Diphenyl Ethers (PBDEs) by Human Hepatocytes in Vitro. *Environ Health Perspect* 2009;117(2):197–202. [PubMed: 19270788] (b) Timme-Laragy AR, Levin ED, Di Giulio RT. Developmental and behavioral effects of embryonic exposure to the polybrominated diphenylether mixture DE-71 in the killifish (*Fundulus heteroclitus*). *Chemosphere* 2006;62(7):1097–1104. [PubMed: 16045967] (c) Wahl M, Lahni B, Guenther R, Kuch B, Yang L, Straehle U, Strack S, Weiss C. A technical mixture of 2,2',4,4'-tetrabromo diphenyl ether (BDE47) and brominated furans triggers aryl hydrocarbon receptor (AhR) mediated gene expression and toxicity. *Chemosphere* 2008;73(2):209–215. [PubMed: 18619640]
13. (a) Boon JP, van Zanden JJ, Lewis WE, Zegers BN, Goksoyr A, Arukwe A. The expression of CYP1A, vitellogenin and zona radiata proteins in Atlantic salmon (*Salmo salar*) after oral dosing with two commercial PBDE flame retardant mixtures: absence of short-term responses. *Mar Environ Research* 2002;54(3–5):719–724. (b) Kuiper R, Bergman A, Vos JG, van den Berg M. Some polybrominated diphenyl ether (PBDE) flame retardants with wide environmental distribution inhibit TCDD-induced EROD activity in primary cultured carp (*Cyprinus carpio*) hepatocytes. *Aquat Toxicol* 2004;68(2):129–139. [PubMed: 15145223]
14. Richardson VM, Staskal DF, Ross DG, Diliberto JJ, DeVito MJ, Bimbaum LS. Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener. *Toxicology and Applied Pharmacology* 2008;226(3):244–250. [PubMed: 17964624]
15. Hakk H, Letcher RJ. Metabolism in the toxicokinetics and fate of brominated flame retardants - a review. *Environ International* 2003;29(6):801–828.
16. Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, Ritchie IJ. Exposure to polybrominated diphenyl ethers (PBDEs): Changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). *Toxicol Sci* 2005;88(2):375–383. [PubMed: 16120752]
17. (a) Black K, Shalat SL, Freeman NCG, Jimenez M, Donnelly KC, Calvin JA. Children's mouthing and food-handling behavior in an agricultural community on the US/Mexico border. *Journal of Exposure Analysis and Environmental Epidemiology* 2005;15(3):244–251. [PubMed: 15292908] (b) Schlenk, D.; Celander, M.; Gallagher, EP.; George, SC.; James, M.; Kullman, SW.; van den Hurk, P.; Willett, K. Biotransformation in Fishes. In: Di Giulio, RT.; Hinton, DE., editors. *The Toxicology of Fishes*. CRC Press; New York: 2008. p. 190-194.
18. Baqui MMA, Gereben B, Harney JW, Larsen PR, Bianco AC. Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy. *Endocrinology* 2000;141(11):4309–4312. [PubMed: 11089566]
19. (a) Harada N, Omura T. Participation of cytochrome P-450 in the reduction of nitro-compounds by rat-liver microsomes. *Journal of Biochemistry* 1980;87(5):1539–1554. [PubMed: 7390998] (b) Nilsen, BM.; Berg, K.; Goksoyr, A. Induction of cytochrome P450 1A (CYP1A) in fish. In: Phillips, IR.; Shephard, EA., editors. *Methods in molecular biology, Cytochrome P450 protocols*. Vol. 107. Humana Press; Totowa: 1998. p. 423-438.
20. Taysse L, Chambras C, Marionnet D, Bosgraud C, Deschaux P. Basal level and induction of cytochrome P450, EROD, UDPGT, and GST activities in carp (*Cyprinus carpio*) immune organs (spleen and head kidney). *B Environ Contam Tox* 1998;60(2):300–305.

21. Stapleton HM, Kelly SM, Allen JG, McClean MD, Webster TF. Measurement of polybrominated diphenyl ethers on hand wipes: Estimating exposure from hand-to-mouth contact. *Environmental Science & Technology* 2008;42(9):3329–3334. [PubMed: 18522114]
22. (a) Hallgren S, Darnerud PO. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats - testing interactions and mechanisms for thyroid hormone effects. *Toxicology* 2002;177(2–3):227–243. [PubMed: 12135626] (b) Zhou T, Taylor MM, DeVito MJ, Crofton KA. Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol Sci* 2002;66(1):105–116. [PubMed: 11861977]
23. Chen D, Mai B, Song J, Sun Q, Luo Y, Zeng EY, Hale R. Polybrominated Diphenyl Ethers in Birds of Prey from Northern China. *Environmental Science & Technology*. 2007 ASAP Alerts.
24. Leonard JL, Rennke H, Kaplan MM, Larsen PR. Sub-cellular distribution of iodothyronine 5'-deiodinase in cerebral-cortex from hypothyroid rats. *Biochimica Et Biophysica Acta* 1982;718(1):109–119. [PubMed: 7138901]
25. Molnar I, Balazs C, Szegedi G, Sipka S. Inhibition of type 2,5'-deiodinase by tumor necrosis factor alpha, interleukin-6 and interferon gamma in human thyroid tissue. *Immunol Lett* 2002;80(1):3–7. [PubMed: 11716958]
26. (a) Fitzsimmons PN, Lien GJ, Nichols JW. A compilation of in vitro rate and affinity values for xenobiotic biotransformation in fish, measured under physiological conditions. *Comp Biochem Phys C* 2007;145(4):485–506. (b) Yahalomi Z, Perry AS. Microsomal Mixed-Function Oxidases in Fish in Relation to Environmental Contamination by Organochlorine Insecticides. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* 1981;70(1):97–102.
27. Yamazaki H, Inoue K, Turvy CG, Guengerich FP, Shimada T. Effects of freezing, thawing, and storage of human liver samples on the microsomal contents and activities of cytochrome p450 enzymes. *Drug Metab Dispos* 1997;25(2):168–174. [PubMed: 9029047]
28. Raldua D, Padros F, Sole M, Eljarrat E, Barcelo D, Riva MC, Barata C. First evidence of polybrominated diphenyl ether (flame retardants) effects in feral barbel from the Ebro River basin (NE, Spain). *Chemosphere* 2008;73(1):56–64. [PubMed: 18597816]
29. Kohrle J. Local activation and inactivation of thyroid hormones: the deiodinase family. *Mol Cell Endocr* 1999;151(1–2):103–119.
30. (a) Frith SD, Eales JG. Thyroid hormone deiodination pathways in brain and liver of rainbow trout, *Oncorhynchus mykiss*. *Gen Comp Endocr* 1996;101(3):323–332. [PubMed: 8729942] (b) Mol K, Kaptein E, Darras VM, Degreef WJ, Kuhn ER, Visser TJ. Different thyroid hormone-deiodinating enzymes in tilapia (*Oreochromis niloticus*) liver and kidney. *Febs Letters* 1993;321(2–3):140–144. [PubMed: 8477843] (c) Orozco A, Valverde-R C. Thyroid hormone deiodination in fish. *Thyroid* 2005;15(8):799–813. [PubMed: 16131323] (d) Orozco A, Linser P, Valverde-R C. Kinetic characterization of outer-ring deiodinase activity (ORD) in the liver, gill and retina of the killifish *Fundulus heteroclitus*. *Comp Biochem Phys B* 2000;126(3):283–290.
31. Sanders JP, VanderGeyten S, Kaptein E, Darras VM, Kuhn ER, Leonard JL, Visser TJ. Characterization of a propylthiouracil-insensitive type I iodothyronine deiodinase. *Endocrinology* 1997;138(12):5153–5160. [PubMed: 9389495]
32. Board PG, Anders MW. Glutathione transferase omega 1 catalyzes the reduction of S-(Phenacyl) glutathiones to acetophenones. *Chem Res Toxicol* 2007;20(1):149–154. [PubMed: 17226937]
33. Mol KA, Van der Geyten S, Burel C, Kuhn ER, Boujard T, Darras VM. Comparative study of iodothyronine outer ring and inner ring deiodinase activities in five teleostean fishes. *Fish Physiol Biochem* 1998;18(3):253–266.
34. Orozco A, Silva JE, Valverde C. Rainbow trout liver expresses two iodothyronine phenolic ring deiodinase pathways with the characteristics of mammalian types I and II 5'-deiodinases. *Endocrinology* 1997;138(1):254–258. [PubMed: 8977411]
35. Nishimura M, Koeda A, Suganuma Y, Suzuki E, Shimizu T, Nakayama M, Satoh T, Narimatsu S, Naito S. Comparison of inducibility of CYP1A and CYP3A mRNAs by prototypical inducers in primary cultures of human, cynomolgus monkey, and rat Hepatocytes. *Drug Metabolism and Pharmacokinetics* 2007;22(3):178–186. [PubMed: 17603218]

Figure 1. Thyroxine (T4) conversion to T3 by deiodinase (DI) enzymes in cells, and similarities to the reductive dehalogenation of BDE-99 to BDE-47 observed *in vitro* and *in vivo* in common carp hepatic tissue.

Figure 2.

In vitro biotransformation rates of 354 pmoles BDE-99 incubated for 60 minutes at 25 °C with microsomal and cytosolic sub-cellular fractions prepared from hepatocytes of 7 adult carp specimens (mean $\pm$ SEM; n=3).

Figure 3.

In vitro biotransformation rates of (A) BDE-47; (B) BDE-66; and (C) BDE-49 (mean $\pm$ SEM; n=3) in pooled carp liver microsomes incubated in 1–250 μ M of BDE-99 for 60 minutes at 25 °C. Concentration of tetraBDE in pmoles hr⁻¹ mg protein⁻¹.

Figure 4. Relative BDE-99 biotransformation rates in pooled carp microsomes measured in the presence of iodoacetate (IaC) (mean $\pm$ SEM; $n=3$). ND indicates not detected. Asterisks indicate data points that were significantly different than controls ($p<0.05$).

Figure 5. BDE-99 biotransformation rates in pooled carp microsomes observed during competitive substrate experiments in the presence of either rT3 or T4 (mean $\pm$ SEM; $n=3$). Asterisks indicate data points that were significantly different than controls ($p<0.05$).

Figure 6.

Proposed *in vitro* biotransformation pathway of meta-, ortho-, and para- cleavage of bromine observed in pooled carp microsomes exposed to (a) 250 μ M of BDE-99 or (b) 125 μ M of BDE-99.

Table 1

Previous studies showing metabolic biotransformation of PBDEs in fish.

Fish Species	Treatment	Exposure	PBDE Metabolism *	Citation
Zebrafish (<i>D. rerio</i>)	BFR mixture, including BDE-28, -183, -209; 1 and 100 nmol/g ww/congener at 2% bw/day	42-day dietary exposure	At 42 days, high dose group: 12 nmol/g ww BDE-149 3 nmol/g ww BDE-154	Nyholm et al., 2009 ^{35,35,34,34}
Chinook salmon (<i>O. tshawytscha</i>)	0.9 µM BDE-99	16-hr incubation, <i>in vitro</i> liver microsomes	2 pmoles BDE-49 hr ⁻¹ mg protein ⁻¹	Browne et al., 2009
Common carp (<i>C. carpio</i>)	12–29 pmoles BDE-99	60-min incubation, <i>in vitro</i> intestine and liver microsomes	BDE-47 formation: 83±34% BDE-99 metabolized in intestine 106±18% BDE-99 metabolized in liver	Benedict et al., 2007
Rainbow trout (<i>O. mykiss</i>)	<i>In vivo</i> : 940 ng BDE-209 at 1% bw/day (trout)	<i>In vivo</i> : 5-month dietary exposure	<i>In vivo</i> : Formation of BDE-188 and other heptaBDEs; BDE-201, -202 and other octaBDEs; BDE-207 and -208; BDE-209 accumulated (trout)	Stapleton et al., 2006
Common carp (<i>C. carpio</i>)	<i>In vitro</i> : 15 pmoles BDE-209 (trout and carp)	<i>In vitro</i> : 24-hr incubation, liver microsomes	<i>In vitro</i> : Formation of octa – nonaBDEs (trout) and hexa – octaBDEs (carp)	
Common carp (<i>C. carpio</i>)	940 ng BDE-209/day/fish	60-day dietary exposure, 40-day depuration	Formation of BDE-154, BDE-155, unknown hexa to octaBDEs; Net formation of hexa – octaBDEs ranged from 0.28–1.03 ng/day; No BDE-209 accumulation	Stapleton et al., 2004a
Common carp (<i>C. carpio</i>)	400 ng BDE-99/day/fish and 100 ng BDE-18/day/fish	62-day dietary exposure, 37 day depuration	Intestinal measures: BDE-47: 9.5% assimilation efficiency BDE-99; BDE-154, other hexaBDE: 17% assimilation efficiency BDE-183	Stapleton et al., 2004b
Common carp (<i>C. carpio</i>)	PBDE mixture, including BDE-99 at 470 ng/day/fish	60-day dietary exposure, 40 day depuration	BDE-47 formation; no measured accumulation of BDE-99 suggesting metabolism	Stapleton et al., 2004c
Lake trout (<i>S. namaycush</i>)	13 PBDE congener mixture at 2.5 and 25 ng/g ww/congener at 1.5% bw/day	56-day dietary exposure, 112 day depuration	Unknown pentaBDE, BDE-140, and unknown hexaBDEs not present in food or control fish Other metabolic products of debromination possible (e.g., BDE-154, -153, -66, -77)	Tomy et al., 2004
Rainbow trout (<i>O. mykiss</i>)	750–1000 ng decaBDE/day/fish	Up to 120-day dietary exposure	Hexa-to nonaBDEs formation (liver) BDE-209 accumulation measured	Kierkegaard et al., 1997

* Reductive metabolites formed may be identified by study authors as “other” or “unknown” homologues if they are measured in tissues but standards are not available to confirm their identity.

Table 2

GST activity in pooled carp liver microsomes incubated with increasing concentrations of rT3.

rT3 (nM)	Activity (nmol CDNB min ⁻¹ mg protein ⁻¹)
0	55.7 ± 6.3
20	70.1 ± 2.8
200	64.4 ± 2.8
1000	60.6 ± 3.9
2000	65.1 ± 1.9