SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 36, No. 8, pp. 2005–2011, 2017
© 2017 SETAC
Printed in the USA

SPECIES-SPECIFIC AND STRUCTURE-DEPENDENT DEBROMINATION OF POLYBROMINATED DIPHENYL ETHER IN FISH BY IN VITRO HEPATIC METABOLISM

YUAN-LAI LUO,[a,b] XIAO-JUN LUO,[a,*] MEI-XIA YE,[a,b] YAN-HONG ZENG,[a] SHE-JUN CHEN,[a] and BI-XIAN MAI[a]

[a]State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Resources Utilization and Protection, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, People's Republic of China

[b]University of Chinese Academy of Sciences, Beijing, People's Republic of China

(Submitted 9 October 2016; Returned for Revision 6 January 2017; Accepted 26 January 2017)

Abstract: To explore the cause of species-specific differences and structure–activity relationships in the debromination of polybrominated diphenyl ethers (PBDEs) in fish, a series of in vitro measurements of hepatic metabolism of PBDE were made using crucian carp (*Carassius carassius*) and catfish (*Silurus asotus*) and the activity of deiodinase in liver microsomes was measured. Debromination was observed in the crucian carp but not in the catfish. No difference was found in total deiodinase activity despite the activity of type 1 deiodinase in crucian carp being twice that of catfish. It is difficult to determine whether the differences in deiodinase activity were responsible for the species-specific differences observed. In crucian carp, penta-brominated diphenyl ether congeners exhibited the highest debromination rates, and the transformation rate decreased with an increasing number of substituted bromines. Adjacent bromine substitution in the phenyl ring was a necessary, but insufficient, condition for debromination in crucian carp. Doubly flanked bromine was always preferentially removed, while single-flanked bromine, meta-substituted bromine, was debrominated the most, followed by para- and then ortho-bromine. No debromination was observed for single-flanked bromine when there was a symmetrical structure with (2, 4, 6) bromine substitutions in 1 phenyl ring, indicating that this structure can improve resistance to debromination metabolism. *Environ Toxicol Chem* 2017;36:2005–2011. © 2017 SETAC

Keywords: Debromination Polybrominated diphenyl ether Structure Fish liver microsome Deiodinase

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants widely used in furniture, textiles, and electrical products [1]. They have proved to be persistent, bioaccumulative, and toxic environmental pollutants [2,3]. Consequently, 2 forms of PBDEs have recently been listed as persistent organic pollutants under the Stockholm Convention [4]. In terrestrial organisms and humans, the main metabolites observed have been hydroxylated brominated diphenyl ethers (BDEs) [5,6], while in fish, debromination is the predominant biotransformation pathway of PBDEs [7]. Debromination of PBDEs in the environment is a concern because of the reaction product congeners have of potentially higher bioaccumulation and toxicity than the parent congeners [8,9]. For this reason, debromination of PBDE has been the subject of intensive study in the past decade.

Several studies have demonstrated that debromination in fish is species-specific. For example, carp was found to metabolically debrominate BDE 99 to a larger extent than Chinook salmon, and the metabolic products differed between species (BDE 47 was formed in carp, but BDE 49 was found in salmon) [10,11]. Both in vivo exposure [12] and field investigations [13] found that cyprinid fish such as common carp, mud carp, and crucian carp can debrominate higher brominated congeners such as BDE 85, 99, 183, 196, 197, 203, 206, 207, 208, and 209 to form lower brominated congeners. However, no debromination was found in siluroid fish such as catfish and plecostomus [14]. To date, little information has been available on the reason for these species-specific differences in PBDE debromination. Noyes et al. [10] suggested that the reductive debromination of PBDEs in fish was related to the relative activity of deiodinase, especially type 1 deiodinase, according to cofactor and substrate competitor experiments. However, no study has so far been conducted to determine whether deiodinase activity is responsible for species-specific debromination of PBDEs. In mammals, 3 types of deiodinase have been investigated, showing that all these enzymes are dependent on thiol cofactors such as dithiothreitol (DTT, which are believed to be selenoenzymes [15]. Type 1 deiodinase exhibits both outer and inner ring deiodination activity and prefers rT3 to T4 and T3 as a substrate. Type 2 deiodinase catalyzes only outer ring deiodination and prefers T4 to rT3. Type 3 deiodinase possesses only inner ring deiodination activity and prefers T3 to T4 as a substrate [16]. In general, information on the properties of deiodinases and their role in PBDE metabolism in teleost fish remains limited and unclear.

Currently, the structure–activity relationship of the debrominated metabolism of PBDE congeners is of particular interest, because it can help us better predict the biotransformation, accumulation, and fate of PBDE congeners in the environment [17], and it may also be vital from a toxicological perspective [18,19]. Roberts et al. [20] investigated the debrominated metabolism of 11 individual PBDE congeners in fish using an in vitro microsome assay, and found that all the congeners that were metabolized contained at least 1 meta-substituted bromine; a metabolite was not detected for congeners without meta-substituted bromine. Zeng et al. [21] investigated the debromination of 3 commercial PBDE mixtures in common carp using the in vivo method. They found that the congeners that contained at least 1 meta- or para- doubly flanked bromine easily underwent metabolic debromination in fish. This result was consistent with the results of the microbial

This article includes online-only Supplemental Data.

* Address correspondence to luoxiaoj@gig.ac.cn

Published online 30 January 2017 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/etc.3749

debromination of PBDE experiments performed by Robrock et al. [22]. However, only a handful of environmentally relevant PBDE congeners were investigated in all the previous studies. Moreover, these studies did not compare metabolic differences between each class of PBDE congeners with common structural characteristics and instead simply described their metabolic pathways, so it was difficult to derive a clear and common law.

In the present study, we conducted a series of in vitro liver microsome assays on crucian carp and catfish to observe the biotransformation of PBDE congeners in different fish species. The role of deiodinase enzymes and their subtypes in the debromination of PBDEs was also evaluated. The debromination processes of 24 individual PBDE congeners (tetra- through deca-) in crucian carp were investigated, and the influence of PBDE structural characteristics (i.e., bromine substitution patterns) on metabolism was evaluated. The results of the present study may improve our understanding of the structure–activity relationship in the PBDE debromination processes.

MATERIALS AND METHODS

Chemicals and reagents

Twenty-four individual PBDE congeners used for dosing (Supplemental Data, Figure S1), quantification standards, surrogate internal standards (BDE 118 and 6-OH-BDE 85), and recovery standards (BDE 77 and 6-MeO-BDE 87) were all purchased from AccuStandard. Dithiothreitol (DTT) and reduced β-nicotinamide adenine dinucleotide 2′-phosphate (NADPH) were obtained from J&K. All other solvents and reagents were of analytical grade or higher and were purchased from standard sources. The PBDE congeners were selected based on the following principles: 1) for tetra- to hexa-PBDE congeners, the bromine substituted position in 1 phenyl ring was fixed as (2, 4) or (2, 4, 6), because no debromination occurred for this structure, according to previous studies [23]; the congeners with all possible structures in another phenyl ring were included; and 2) for hexa-BDE congeners without (2, 4, 6) structure to deca-BDE, environmentally relevant congeners were chosen.

Preparation of hepatic subcellular fractions

Ten crucian carp (*Carassius carassius*) with an average weight of 352.2 ± 26.3 g and length of 23.5 ± 0.5 cm, and 10 catfish (*Silurus asotus*) with a mean weight of 605.5 ± 39.2 g and length of 34.8 ± 1.7 cm were purchased from the aquarium market in Guangzhou, China. The fish were euthanized by a sharp blow to the head. The livers were quickly removed, rinsed with ice-cold 0.9% NaCl solution, and weighed. The liver tissues pooled from individual fish of each species were homogenized in a 0.15 M potassium chloride-phosphate–buffered saline. Microsome fractions were isolated using standard methods [24] and typically included DTT in the homogenization, wash, and resuspension buffers, to preserve the catalytic activity of reductases and deiodinases. The microsome suspension was divided into aliquots and stored at –80 °C until it was utilized for in vitro assays. The protein content analysis of subcellular fractions was based on previously reported methods [25]. Analytical methods and the results of enzymatic activity of subcellular fractions, consisting of cytochrome P450 and deiodinase assays are provided in the Supplemental Data.

In vitro incubation assay

Reaction mixtures (1 mL) consisting of 100 mM phosphate buffer (pH 7.4), pooled fish liver microsomes, and individual PBDE samples (1 nmol) were prepared on ice. The samples were preincubated for 5 min in a shaking water bath at 25 °C. The reaction was initiated by the addition of DTT and NADPH (10 mM and 1 mM, respectively, final concentration), and quenched by adding 0.1 mL of methanol containing 5% formic acid in volume and the surrogate standards (60 ng BDE 118 and 30 ng 6-OH-BDE 85 used for PBDE metabolism assay) after incubation for 4 h. All incubations were conducted along with the nonenzymatic controls without DTT and NADPH blanks and additional blanks containing a carrier in lieu of the substrate [26]. Previous studies have shown that higher substrate concentrations result in a wider variety and higher abundance of metabolites [10]. However, in the present study, we chose a lower substrate concentration (1 μM) to more closely resemble environmentally relevant concentrations, yet still generating detectable levels of metabolites.

Sample extraction

The extraction of PBDEs followed methods similar to those of our previous studies [21]. Samples were vortex mixed for 30 s and then extracted twice with methyl *tert*-butyl ether:hexane (1:1, v/v, 4 mL and 2 mL). The combined organic extracts were dissolved with hexane and partitioned with potassium hydroxide solution (0.5 N in 50% ethanol). The neutral fraction used for PBDE analysis was treated with 1 mL concentrated sulfuric acid to remove lipids, cleaned on a multilayer silica gel column, and then eluted with 20 mL dichloromethane:hexane (1:1, v/v). Finally, the extract was blown to near dryness under nitrogen and redissolved in isooctane adjusted to 300 μL. Recovery standard BDE 77 was added before gas chromatography/mass spectrometry (GC/MS) analysis. The alkaline phase used for OH-BDE analysis was acidified with HCl to $pH < 2$, then extracted twice with methyl *tert*-butyl ether:hexane (1:1, v/v, 4 mL and 2 mL), and dried using anhydrous sodium sulfate. Samples were derivatized with diazomethane overnight to methylate the hydroxyl group. After methylation, the samples were purified on a silica gel column (inner diameter 0.8 cm) packed with 1 cm neutral silica (3% deactivated, w/w) and 7 cm sulfuric acid silica (2:1 w/w), eluted with 15 mL hexane and 5 mL dichloromethane:hexane (1:1, v/v). The extract was blown to near dryness and reconstituted in 300 μL isooctane. Then 6-MeO-BDE 87 was added as a recovery standard for GC/MS analysis.

Instrumental analysis

Tri- to hepta-BDE and OH-PBDE (derivatized to MeO-PBDE) congeners were analyzed by GC/MS (Agilent 7890A/5975B MSD) with an electron capture negative ionization source in a selective ion monitoring mode, and their separation was performed using a DB-XLB capillary column (30 m × 0.25 mm inner diameter × 0.25 μm film thickness). For quantification of octa- to deca-BDEs, a DB-5HT capillary column (15 m × 0.25 mm inner diameter × 0.1 μm film thickness) was used to separate the analytes. The oven temperature procedure is described in detail in the Supplemental Data.

All analytes were identified based on their retention times on the capillary columns and verified by matching retention times with authentic standard mixtures (39 PBDE congeners, obtained from AccuStandards). Quantification was performed using the internal calibration method, based on a 5-point calibration curve. Congeners that were not included in the authentic standards (39 congeners) were quantified using the relative response factors to the individual BDE authentic standard.

Quality assurance and data analysis

The average recoveries of BDE 118 and 6-OH-BDE 85 in the microsome extracts were $102 \pm 6\%$ and $87 \pm 5\%$, respectively. Samples with active microsomes were blank corrected for low concentrations of impurities detected in spiking solutions observed in incubations with control samples. The limit of detection was defined as a signal-to-noise ratio greater than 3. All the values were expressed as the mean $\pm$ standard deviation. Statistical analysis of the data was performed in SPSS 19.0 with differences considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Comparison metabolism of PBDEs in crucian carp and catfish

In vitro incubation experiments of 3 penta-BDEs congeners (BDE 99, BDE 85, and BDE 123), with 2 species of fish hepatic microsomes, were carried out in the present study. As expected, obvious debromination metabolism with different metabolic products and rates, was observed in crucian carp for all 3 congeners (Table 1), whereas no debromination was observed in catfish. This result is consistent with that of previous in vivo

Table 1. Debrominated metabolism of individual brominated diphenyl ether (BDE) congeners (1 nmol) by crucian carp liver microsomes ($n = 3$, 25 °C, 4 h)

Parent	Metabolite	[Br] position[a]	Yield (pmols)	Proportion (%)	Formation rate (pmol h^{-1} mg Protein^{-1})
Tetra-BDEs					
42	17	m	32.5 ± 3.8	100.0	20.3
66	28	m	47.6 ± 6.1	67.7	29.8
	25	p	22.7 ± 4.7	32.3	14.2
68	ND[b]	—	—	—	—
47	ND[b]	—	—	—	—
49	ND[b]	—	—	—	—
51	ND[b]	—	—	—	—
Penta-BDEs					
85	47	m	201.4 ± 30.2	94.4	125.9
	42/66[c]	p/o	12.2 ± 2.6	5.6	7.6
123	68	p	210.8 ± 25.3	66.3	131.8
	66	m	108.0 ± 17.6	33.7	67.5
99	47	m	241.8 ± 35.5	91.7	151.1
	49	p	22.0 ± 4.8	8.3	13.8
90	49/68[c]	m/o	178.3 ± 26.4	100.0	111.4
91	51	m	135.8 ± 15.6	87.5	84.9
	49	o	19.4 ± 3.1	12.5	12.1
100	ND[b]	—	—	—	—
Hexa-BDEs					
140	100	m	58.1 ± 3.9	84.6	36.3
	119	o	10.6 ± 1.5	15.4	6.6
168	121	p	34.2 ± 2.1	65.3	21.4
	119	m	18.2 ± 1.3	34.7	11.4
154	ND[b]	—	—	—	—
148	ND[b]	—	—	—	—
150	ND[b]	—	—	—	—
155	ND[b]	—	—	—	—
138	47	m,m	92.6 ± 5.2	91.7	57.9
	99	m	5.7 ± 0.6	5.6	3.2
	85	m	2.7 ± 0.5	2.7	1.7
153	47	m,m	30.9 ± 2.3	67.6	19.3
	101	p	12.7 ± 1.7	27.8	7.9
	99	m	2.1 ± 0.3	4.6	1.3
Hepta-BDE					
183	154	m	62.2 ± 7.7	83.6	38.9
	149	p	7.8 ± 0.9	10.5	4.9
	153	o	4.4 ± 0.2	5.9	2.8
Octa-BDE					
197	184	m	24.9 ± 1.3	87.4	15.6
	155	m,m	2.5 ± 0.3	8.8	1.6
	176	p	1.1 ± 0.1	3.9	0.7
Nona-BDE					
207	197	m	9.7 ± 0.8	39.4	6.1
	201	p	8.7 ± 0.9	29.6	5.4
	200/203[c]	p/o	4.6 ± 0.6	18.7	2.9
	204	m	1.0 ± 0.4	4.1	0.6
	184	m,m	0.6 ± 0.3	2.4	0.4
Deca-BDE					
209	207	m	3.4 ± 0.7	45.3	2.1
	208	p	1.7 ± 0.5	22.7	1.1
	197	m,m	1.3 ± 0.2	17.3	0.8
	200/203[c]	m,p/m,o	0.6 ± 0.1	8.0	0.4
	201	m,p	0.5 ± 0.1	6.7	0.3

[a] m, p, and o in the [Br] position column indicate meta-, para-, and ortho-, respectively.
[b] ND indicates that nothing was detected.
[c] Indicates the 2 BDE congeners coelute with each other.

experiments and field investigations, indicating distinct species-specific differences in the metabolism of PBDEs [27,28].

Previous studies have ruled out the likely involvement of cytochrome P450s [11,29] and suggested that deiodinase may play a role in debromination [30,31], which our results further confirmed. The absence of NADPH did not affect the capacity of crucian carp microsomes to debrominate BDE 99 (Table 2). In contrast, omission of the reductant DTT resulted in either no debromination or a great decrease in the transformation of BDE 99 to 47, suggesting a potentially more prominent role of reductases such as deiodinase in catalyzing this reaction. In addition, it is worth mentioning that the phase II metabolizing enzymes uridinediphosphate-glucuronosyltransferases (UGTs) and glutathione-*S*-transferases (GSTs) have also been suggested to play a role in PBDE metabolism via conjugation processes [10]. However, our incubation system does not contain the cofactor uridinediphosphate glucuronic acid, and it seems unlikely that UGTs are mediating the biotransformation of BDE 99 in crucian carp. With regard to GST, previous study has suggested that GSTs are not involved in PBDE debromination in fish [11,20]. Therefore, these 2 metabolic pathways in fish microsomes seem unlikely.

The total deiodinase activities measured via enzyme-linked immunosorbent assays (ELISAs) were 15.58 ± 0.57 U/L for crucian carp and 15.33 ± 0.48 U/L for catfish. The activities of type 1, type 2, and type 3 deiodinase were 4.17 ± 0.22, 6.59 ± 0.30, and 4.61 ± 0.26 U/L for crucian carp, and 2.02 ± 0.19, 9.18 ± 0.35, and 3.90 ± 0.28 U/L for catfish, respectively. The type 1 deiodinase activity of crucian carp was more than 2 times that of catfish. Noyes et al. [10] suggested that type 1 deiodinase played a more prominent role in catalyzing the debromination of PBDEs in common carp. To some extent, this seems consistent with the results of the present study. However, it is hard to believe that no debromination occurring in catfish was the result of the activity of type 1 deiodinase being half that in crucian carp alone. This raised the question of whether the significant species-specific differences in the debromination between crucian carp and catfish was the result of the deiodinase activities of 2 fish species.

A possible explanation is that the deiodinase in catfish differ from those in crucian carp, but this difference cannot be characterized by ELISA, because the mechanism of the ELISA method is different from that of debromination of PBDEs. The transformation of rT3 to T2 was mediated mainly by type 1 deiodinase [16]. The study of Mol et al. [16] indicated that the transformation of rT3 to T2 was not observed in liver of catfish but was observed in liver of 3 other fish species, tilapia, trout, and turbot. In our previous studies [14,23], tilapia was found to have the same debromination metabolism for PBDEs as carp. On the other hand, the transformation of T4 to T3, mainly mediated by type 2 deiodinase, was observed in the livers of both catfish and 3 other fish species. Therefore, we supposed that the debromination of PBDEs has the same mechanism as that of rT3 to T2. This means that type 1 deiodinase activity determines the debromination of PBDEs. However, it has been pointed out repeatedly in previous studies that these indirect proofs cannot rule out the possibility that other enzyme systems could also be responsible for the debromination of PBDEs in fish. Further studies using purified deiodinase need to be conducted to confirm the involvement of deiodinase in PBDE debromination [20].

Table 2. Influence of cofactors on debromination of brominated diphenyl ether (BDE) 99 to tetra-brominated congeners by crucian carp liver microsomes ($n = 3$)

	Metabolites (pmol)	
Incubation conditions[a]	BDE 47	BDE 49
+NADPH, +DTT	241.8 ± 35.5	22.0 ± 3.1
+NADPH, –DTT	10.3 ± 1.7	ND[b]
–NADPH, +DTT	246.9 ± 27.4	24.5 ± 2.8
–NADPH, –DTT	ND[b]	ND[b]

[a]Incubation conditions were described in detail in the *Materials and Methods* section. reduced β-nicotinamide adenine dinucleotide 2′-phosphate and dithiothreitol were the cofactors of cytochrome P450 and deiodinase, respectively.
[b]ND indicates not detected.
BDE = brominated diphenyl ether; NADPH = reduced β-nicotinamide adenine dinucleotide 2′-phosphate; DTT = dithiothreitol.

Oxidative metabolism of PBDE congeners is also an important biotransformation pathway in some marine species such as algae, fish, and birds [32,33], as well as terrestrial organisms and humans [34]. Our previous in vivo study also confirmed the hydroxylation of PBDE in carp, but at very low levels [21]. Shen et al. [26] observed oxidative metabolites of BDE 15, but not BDE 47 in in vitro treatments of BDE 15 and BDE 47 using carp liver microsomes. However, no obvious oxidative metabolite was detected in crucian carp or catfish in the present study. This may be because the liver microsomes of these 2 freshwater fish had no oxidative metabolic ability for penta-BDEs, or else the metabolic rate was so low that apparent oxidative metabolism could not be observed following the substrate concentration and incubation time under this experimental condition. Alternatively, the results of the present study indicated that in vitro experiments cannot completely reflect the in vivo situation.

Debromination of PBDEs in crucian carp

Of the 24 PBDE congeners tested, 16 were found to debrominate and form lower brominated BDE congeners. Details on the parent compounds and products are provided in Table 1 and Figure 1. The most rapidly metabolized congeners were BDE 123, with a transformation rate of 199 pmol h^{-1} mg protein^{-1} and BDE 99 (165 pmol h^{-1} mg protein^{-1}), and the slowest metabolized congener was BDE 209 (4.7 pmol h^{-1} mg protein^{-1}). Generally, penta-BDE congeners exhibited the highest transformation rates among all PBDE congeners metabolized, and a decreased trend of the transformation rate with increasing substituted bromine atoms was observed (Figure 2). The debromination potential of PBDE congeners increased as the number of substituted bromine increased (tetra- to penta-BDEs). This could be because of the increased octanol/water partition coefficient K_{OW} values from tetra- to penta-BDEs as the bromine atoms increase. The penta-BDEs show stronger lipophilicity and hydrophobicity, and are more likely to bind to enzyme proteins, thereby exhibiting greater metabolic rates than tetra-BDEs in general. However, superabundant bromine atom substitution (hexa- to deca-BDEs) will cause steric hindrance because of the larger molecular volume, which makes it more difficult for the substrate to combine with enzymes, consequently reducing the possibility of a reaction. This could be why the highest metabolic rates were observed in penta-BDE congeners.

Among the 6 tetra-BDE congeners, only 2 congeners (BDE 42 and 66) were found to debrominate to form tri-BDE congeners (BDE 17, 28, and 25). Five of the 6 penta-BDE congeners were debrominated to form a variety of

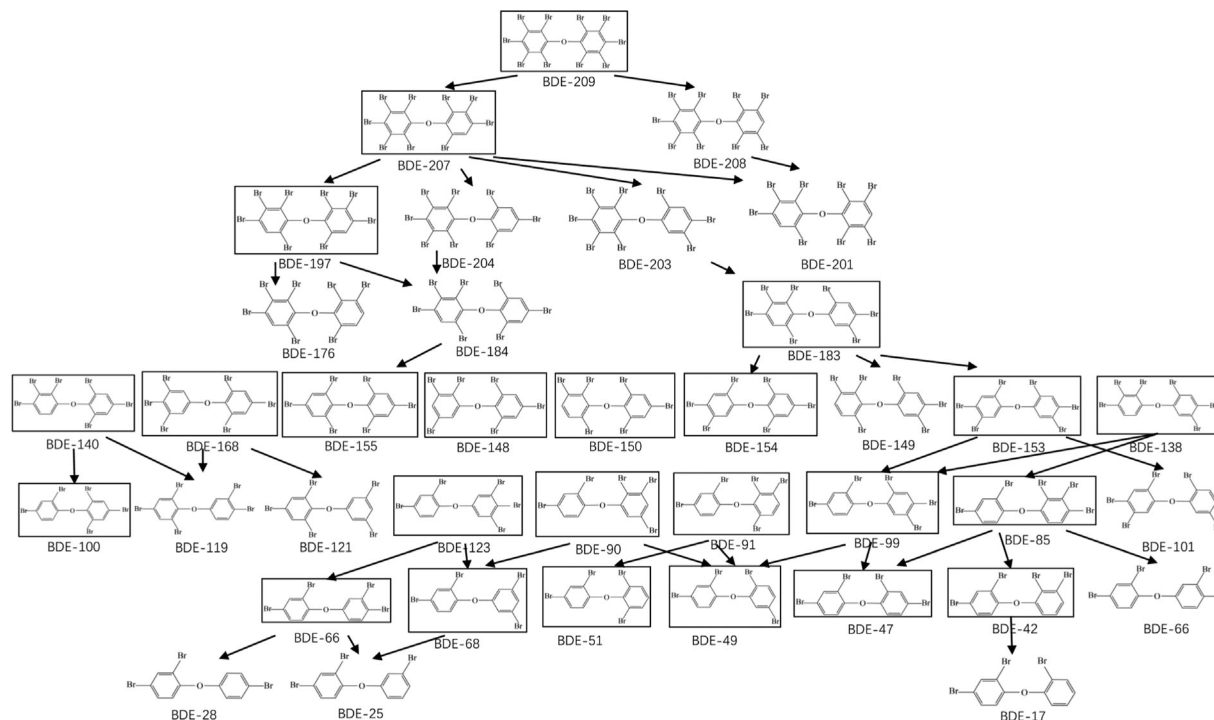

Figure 1. Debromination pathway of all tested brominated diphenyl ether (BDE) congeners by crucian carp hepatic microsomal biotransformation (25 °C, 4 h). Boxes indicate congeners used as initial substrates in incubations. Arrows indicate the formation of substrates to metabolites. All metabolized debromination in the present study occurred in the adjacent bromine atoms. BDE 200 coelutes with BDE 203, which is also a potential metabolite not shown.

tetra-BDE metabolites. No metabolism was observed in incubations with BDE 100. The congener with the greatest degree of metabolism in the present study was BDE 123 (319 pmol), The main products of BDE 123 were BDE 68 (para removal) and BDE 66 (meta removal), which is also not consistent with the metabolism of BDE 99 primarily by meta removal [10]. The extent of metabolism of the 5 penta-BDE congeners followed the order: BDE 123 (319 pmol) > BDE 99 (264 pmol) > BDE 85 (214 pmol) > BDE 90 (178 pmol) > BDE 91 (155 pmol).

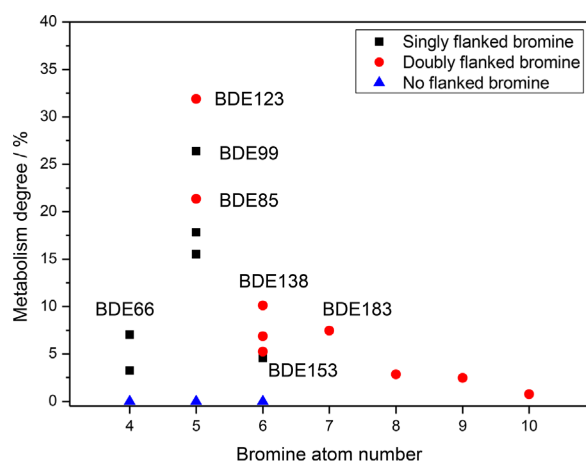

Figure 2. Relationship between the degree of metabolism of individual brominated diphenyl ether (BDE) congeners and the number of bromine atoms. Three different bromine-substituted structures of all the tested BDEs are also indicated. The BDEs without any metabolism in the figure were the congeners without flanked bromine (contain tetra-BDEs: BDE 47, 49, 51, 68; penta-BDE 100) and hexa-BDEs with (2, 4, 6) structure (contain BDEs 148, 150, 154, 155).

In the 6 hexa-BDE congeners with (2, 4, 6) bromine substitution in a phenyl ring, metabolic debromination was only observed in BDEs 140 and 168. The main products were BDEs 100, 121, and 119. The other 2 hexa-BDE congeners without the (2, 4, 6) substitution structure, BDEs 138 and 153, were debrominated to form mainly BDE 47. A few intermediates, BDEs 99 and 85, were also detected in the incubations. Besides BDE 47, BDE 101 was also observed as a minor product of BDE 153.

As for other congeners (hepta- to deca-BDE), the metabolic products and debromination pathways in the present study were similar to those found in a study by Roberts et al. [20] using common carp liver microsomes. In addition, the debromination from BDE 99 to BDE 47 was the most rapid transformation observed in the present study, which is also consistent with the observation of Roberts et al. [20]. This indicated that crucian carp had the same metabolic mechanisms for the debromination of PBDEs.

Structure-dependent debromination

All the PBDE congeners debrominated to some extent by crucian carp hepatic microsomes in the present study have a common structural feature—adjacent bromines. No debromination was observed for PBDE congeners without adjacent bromines. There are 2 types of adjacent bromines: singly flanked and doubly flanked bromine. The doubly flanked meta- or para-bromines were always removed at a faster rate than the singly flanked bromines. This can be verified by the debromination of BDE 85, BDE 123, BDE 140, BDE 168, BDE 138, and BDE 183 (Figure 2). For example, in the debromination of BDE 85, the formation rate of the removal of doubly flanked meta-bromine (to form BDE 47) was approximately 20 times that of singly flanked para- or ortho-bromine (to form BDE 42/66). The congener BDE 138 can remove the

doubly flanked meta-bromine (2, 3, 4- in 1 phenyl ring) to form BDE 99, and it can also remove the singly flanked meta-bromine (2′, 4′, 5′- in another phenyl ring) to form BDE 85. Although the transformation rate of BDE 99 was faster than that of BDE 85, the remaining mass of BDE 99 was still 2 times that of BDE 85, indicating a faster removal rate for doubly flanked bromine than singly flanked bromine. The same situation was found in 4 other congeners (Table 1). Given that repulsion between adjacent bromines results in increased enthalpies of formation, it is not surprising that doubly flanked bromines are removed more frequently than singly flanked bromines [35]. Similar results were also reported for the microbial debromination of PBDEs [22] and the in vivo experiments of common carp exposure to 3 PBDE technical mixtures [21].

With regard to singly flanked bromines, 2 structures were considered: ortho-meta and meta-para. Regardless of the structure, the meta-substituted bromine atom was always preferentially removed. For example, the formation rate of BDE 28 (removal of the meta-bromine) was 2 times that of BDE 25 (removal of the para-bromine) by in vitro incubations with BDE 66 (Table 1). Meanwhile, the formation of BDE 51 by the removal of meta-bromine of BDE 91 occurred 7 times more rapidly than the formation of BDE 49 by the removal of ortho-bromine of BDE 91. A similar situation was also found in the debromination of BDE 42 and BDE 99 (Table 1). As can be seen in Figure 3, meta-debromination of PBDEs is a dominant metabolic pathway for the biotransformation of all PBDE homologues in crucian carp. In their study of the debromination of PBDEs in carp, Roberts et al. [20] obtained similar results: common carp demonstrated a clear preference for meta-debromination. However, compared with the biotransformation in fish, photobromination experiments showed a different trend, with ortho- and meta-substitutions as the dominant metabolic approaches [15,35].

In the present study, not all adjacent bromines in the phenyl ring could undergo debromination. The BDEs 154, 148, and 150 all had adjacent bromines (meta-ortho and meta-para), but no metabolites were observed. This result indicated that a structure with adjacent bromine is a necessary but insufficient factor. Other structural factors can affect debromination. When comparing the structures of BDEs 153 and 154, we found that the difference between the 2 chemicals was that BDE 154 had a (2, 4, 6) bromine substituted structure whereas BDE 153 had a (2, 4, 5) bromine substituted structure. Similarly, BDE 138 had the same structure (2, 3, 4) in 1 phenyl ring, but a different structure in another phenyl ring (2, 4, 5 vs 2, 4, 6) compared with BDE 140. The transformation rate of BDE 140 was lower than that of BDE 138 (Table 2). These results led to the assumption that the structure (2, 4, 6) in 1 phenyl ring can significantly reduce the debromination potential of bromine in another phenyl ring, especially for single adjacent bromines. The symmetric structure of a (2, 4, 6) substitution might improve the stability of PBDEs [35,36], which could be a reason for the relatively high abundance of BDE 154 in the field fish samples. More experiments are needed to confirm this issue using pairs of congeners with a specific structure (i.e., BDEs 146/148, and BDEs 149/150).

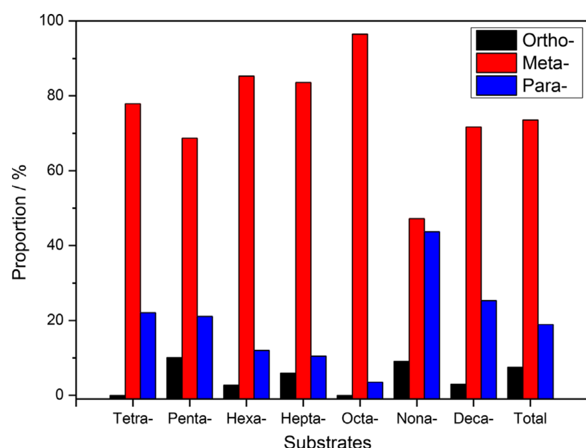

Figure 3. Relative proportions of different debromination positions for each brominated diphenyl ether (BDE) homologue and total BDE congeners by crucian carp hepatic microsomal biotransformation. The calculation of relative proportion is based on the sum of formation rates of all metabolites formed based on the debromination position.

CONCLUSIONS

The present study demonstrated the significant species-specific biotransformation of PBDE congeners in crucian carp and catfish. Although some differences were observed in the activities of deiodinase between the 2 fish species, there is still not enough evidence to support the hypothesis that deiodinase are responsible for the species differences in metabolic debromination observed. An understanding of the difference in activities of deiodinase between different fish species is critical for understanding their role in the debromination of PBDEs. Other reductases might also be responsible for the debromination of PBDEs. Compared with penta-BDEs, the debrominated metabolic rates were significantly reduced with increases or decreases of substituted bromine numbers. The results of the present study indicate that all debromination involved the removal of bromine atoms that were adjacent to at least 1 other bromine. Doubly flanked bromine was always preferentially removed. In singly flanked bromine, the removal of the meta-position to the para- and ortho-position was preferred. The (2, 4, 6) bromine substituted structure in 1 phenyl ring greatly reduced the debromination potential of bromine in the other ring, especially in singly flanked bromine. Understanding these characteristics of the biotransformation of PBDE congeners is important in predicting their environmental fate, and may also be vital from a toxicological perspective.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3749.

Acknowledgment—The present study was supported financially by the National Basic Research Program of China (2015CB453102), the National Nature Science Foundation of China (41273118, 41473102, 41673100, and 41230639), and the Science and Technology Project of Guangdong Province, China (2014B030301060). This is contribution No. IS-2346 and SKLOGA201602 from GIGCAS.

Data Availability—Data for the present study can be obtained by contacting the corresponding author (luoxiaoj@gig.ac.cn).

REFERENCES

1. de Wit CA. 2002. An overview of brominated flame retardants in the environment. *Chemosphere* 46:583–624.
2. Crump D, Chiu S, Egloff C, Kennedy SW. 2008. Effects of hexabromocyclododecane and polybrominated diphenyl ethers on mRNA expression in chicken (*Gallus domesticus*) hepatocytes. *Toxicol Sci* 106:479–487.
3. Fernie KJ, Shutt JL, Letcher RJ, Ritchie IJ, Bird DM. 2009. Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. *Environ Sci Technol* 43:2124–2130.
4. Ross PS, Couillard CM, Ikonomou MG, Johannessen SC, Lebeuf M, Macdonald RW, Tomy GT. 2009. Large and growing environmental

reservoirs of deca-BDE present an emerging health risk for fish and marine mammals. *Mar Pollut Bull* 58:7–10.
5. Liu J, Luo XJ, Yu LH, He MJ, Chen SJ, Mai BX. 2010. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyles (PCBs), hydroxylated and methoxylated-PBDEs, and methylsulfonyl-PCBs in bird serum from south China. *Arch Environ Contam Toxicol* 59:492–501.
6. Qiu XH, Bigsby RM, Hites RA. 2009. Hydroxylated metabolites of polybrominated diphenyl ethers in human blood samples from the United States. *Environ Health Perspect* 117:93–98.
7. Stapleton HM, Alaee M, Letcher RJ, Baker JE. 2004. Debromination of the flame retardant decabromodiphenyl ether by juvenile carp (*Cyprinus carpio*) following dietary exposure. *Environ Sci Technol* 38:112–119.
8. McDonald TA. 2002. A perspective on the potential health risks of PBDEs. *Chemosphere* 46:745–755.
9. Darnerud PO. 2003. Toxic effects of brominated flame retardants in man and in wildlife. *Environ Int* 29:841–853.
10. Noyes PD, Kelly SM, Mitchelmore CL, Stapleton HM. 2010. Characterizing the in vitro hepatic biotransformation of the flame retardant BDE 99 by common carp. *Aquat Toxicol* 97:142–150.
11. Browne EP, Stapleton HM, Kelly SM, Tilton SC, Gallagher EP. 2009. In vitro hepatic metabolism of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE 99) in Chinook salmon (*Onchorhynchus tshawytscha*). *Aquat Toxicol* 92:281–287.
12. Zeng YH, Yu LH, Luo XJ, Chen SJ, Wu JP, Mai BX. 2013. Tissue accumulation and species-specific metabolism of technical pentabrominated diphenyl ether (DE-71) in two predator fish. *Environ Sci Technol* 32:757–763.
13. Zeng YH, Luo XJ, Yu LH, Chen HS, Wu JP, Chen SJ, Mai BX. 2013. Using compound-specific stable carbon isotope analysis to trace metabolism and trophic transfer of PCBs and PBDEs in fish from an e-waste site, South China. *Environ Sci Technol* 47:4062–4068.
14. Sun RX, Luo XJ, Tang B, Li ZR, Wang T, Tao L, Mai BX. 2016. Persistent halogenated compounds in fish from rivers in the Pearl River Delta, South China: Geographical pattern and implications for anthropogenic effects on the environment. *Environ Res* 146:371–378.
15. Croteau W, Whitemore SL, Schneider MJ, St. Germain DL. 1995. Cloning and expression of a cDNA for a mammalian type III iodothyronine deiodinase. *J Biol Chem* 270:16569–16575.
16. Mol KA, Van der Geyten S, Burel C, Kühn ER, Boujard T, Darras VM. 1998. Comparative study of iodothyronine outer and inner ring deiodinase activities in five teleostean fishes. *Fish Physiol Biochem* 18:253–266.
17. Zou Y, Christensen ER, Wei Z, Hua W, An L. 2014. Estimating stepwise debromination pathways of polybrominated diphenyl ethers with an analogue Markov Chain Monte Carlo algorithm. *Chemosphere* 114:187–194.
18. Erratico CA, Szeitz A, Bandiera SM. 2013. Biotransformation of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) by human liver microsomes: Identification of cytochrome P450 2B6 as the major enzyme involved. *Chem Res Toxicol* 26:721–731.
19. Erratico C, Zheng X, Ryden A, Marsh G, Maho W, Covaci A. 2015. Human hydroxylated metabolites of BDE-47 and BDE-99 are glucuronidated and sulfated in vitro. *Toxicol Lett* 236:98–109.
20. Roberts SC, Noyes PD, Gallagher EP, Stapleton HM. 2011. Species-specific differences and structure-activity relationships in the debromination of PBDE congeners in three fish species. *Environ Sci Technol* 45:1999–2005.
21. Zeng YH, Luo XJ, Chen HS, Yu LH, Chen SJ, Mai BX. 2012. Gastrointestinal absorption, metabolic debromination, and hydroxylation of three commercial polybrominated diphenyl ether mixtures by common carp. *Environ Toxicol Chem* 31:731–738.
22. Robrock KR, Korytár P, Alvarez-Cohen L. 2008. Pathways for the anaerobic microbial debromination of polybrominated diphenyl ethers. *Environ Sci Technol* 42:2845–2852.
23. Luo XJ, Zeng YH, Chen HS, Wu JP. 2013. Application of compound-specific stable carbon isotope analysis for the biotransformation and trophic dynamics of PBDEs in a feeding study with fish. *Environ Pollut* 176:36–41.
24. Mckinney MA, Arukwe A, Guise SD, Martineau D, Béland P, Dallaire A, Lair S, Lebeuf M, Letcher RJ. 2004. Characterization and profiling of hepatic cytochromes P450 and phase II xenobiotic-metabolizing enzymes in beluga whales (*Delphinapterus leucas*) from the St. Lawrence River Estuary and the Canadian Arctic. *Aquat Toxicol* 69:35–49.
25. Bradford M. 1976. A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254.
26. Shen MN, Cheng J, Wu RH, Zhang SH, Mao L, Gao SX. 2012. Metabolism of polybrominated diphenyl ethers and tetrabromobisphenol A by fish liver subcellular fractions in vitro. *Aquat Toxicol* 114–115:73–79.
27. Sun RX, Luo XJ, Tan XX, Tang B, Li ZR, Mai BX. 2015. An eight year (2005–2013) temporal trend of halogenated organic pollutants in fish from the Pearl River Estuary, South China. *Mar Pollut Bull* 93:61–67.
28. He MJ, Luo XJ, Chen MY, Sun YX, Chen SJ, Mai BX. 2012. Bioaccumulation of polybrominated diphenyl ethers and decabromodiphenyl ethane in fish from a river system in a highly industrialized area, South China. *Sci Total Environ* 419:109–115.
29. Benedict RT, Stapleton HM, Letcher RJ, Mitchelmore CL. 2007. Debromination of polybrominated diphenyl ether-99 (BDE-99) in carp (*Cyprinus carpio*) microflora and microsomes. *Chemosphere* 69: 987–993.
30. Stapleton HM, Letcher RJ, Li J, Baker JE. 2004. Dietary accumulation and metabolism of polybrominated diphenyl ethers by juvenile carp (*Cyprinus carpio*). *Environ Toxicol Chem* 23:1939–1946.
31. Jarque S, Piña B. 2014. Deiodinases and thyroid metabolism disruption in teleost fish. *Environ Res* 135:361–375.
32. Malmvärn A, Zebühr Y, Kautsky L, Bergman Å, Asplund L. 2008. Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-p-dioxins in red alga and cyanobacteria living in the Baltic Sea. *Chemosphere* 72:910–916.
33. Olsson A, Ceder K, Bergman Å, Helander B. 2000. Nestling blood of the white-tailed sea eagle (*Haliaeetus albicilla*) as an indicator of territorial exposure to organohalogen compounds—An evaluation. *Environ Sci Technol* 34:2733–2740.
34. Athanasiadou M, Cuadra SN, Marsh G, Bergman A, Jakobsson K. 2008. Polybrominated diphenyl ethers (PBDEs) and bioaccumulative hydroxylated PBDE metabolites in young humans from Managua, Nicaragua. *Environ Health Perspect* 116:400–408.
35. Zeng X, Simonich SLM, Robrock KR, Korytár P, Alvarez-Cohen L, Barofsky DF. 2010. Application of a congener-specific debromination model to study photodebromination, anaerobic microbial debromination, and FE^0 reduction of polybrominated diphenyl ethers. *Environ Toxicol Chem* 29:770–778.
36. Wang Y, Li A, Liu H, Zhang Q, Ma W, Song W, Jiang G. 2006. Development of quantitative structure gas chromatographic relative retention time models on seven stationary phases for 209 polybrominated diphenyl ether congeners. *J Chromatogr A* 1103:314–328.