DIETARY ACCUMULATION AND METABOLISM OF POLYBROMINATED DIPHENYL ETHERS BY JUVENILE CARP (CYPRINUS CARPIO)

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Abstract—Polybrominated diphenyl ethers (PBDEs) are hydrophobic organic contaminants with properties and nomenclature similar to polychlorinated biphenyls (PCBs). While much information is available on the bioaccumulation and pharmacokinetics of PCBs, little information is available on PBDEs. In this study, juvenile carp were exposed to a diet spiked with a cocktail of four BDE congeners (2,4,4′-tribromoDE [BDE 28], 2,2′,4,4′-tetrabromoDE [BDE 47], 2,2′,4,4′,5-pentabromoDE [BDE 99], and 2,2′,4,4′,5,5′-hexabromoDE [BDE 153]) for 60 d followed by a 40-d depuration period. As a positive control, three PCB congeners with similar log Kow values (2,2′,5,5′-tetrachlorobiphenyl [PCB 52], 2,2′,4,4′, 5,5′-hexachlorobiphenyl [PCB 153], and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl [PCB 180]) were included in the cocktail to compare their assimilation and fate with the model BDE congeners. Concentrations of BDEs and PCBs were monitored in whole-fish tissues and liver tissues over the duration of the experiment. In addition, blood serum samples were taken and pooled among replicates to determine if any phenolic metabolites of BDE and PCBs were formed. Rapid assimilation of BDE 47 was observed relative to all other BDE and PCB congeners, whereas apparently no accumulation of BDE 99 occurred over the course of the experiment. Assimilation efficiencies for BDE 47 suggest that approximately 100% of the BDE 47 exposure was absorbed by carp tissues after 60 d. However, based on the time course of BDE 47 assimilation, it is improbable that all BDE 47 was assimilated; more likely, production of BDE 47 in carp tissues occurred as a result of debronymination of higher-brominated compounds, possibly BDE 99. The net assimilation efficiencies of BDE 28 and BDE 153 were also apparently low (20 and 4%, respectively) relative to the three PCBs (40% assimilated) examined in this study. The low assimilation efficiency and high depuration rates for BDEs suggest a higher potential for biotransformation. While all three PCB compounds displayed very similar assimilation and depuration rates, three of the four BDE compounds displayed significantly different assimilation rates among BDE congeners and relative to the PCBs. This study suggests that BDEs have significantly different fate dynamics relative to PCBs in wild carp and likely other species of fish.

Keywords—Polybrominated diphenyl ethers Polychlorinated biphenyl Biotransformation Metabolism Carp

INTRODUCTION

Over the past decade, concern has been mounting over the increasing levels of the flame retardants polybrominated diphenyl ethers (PBDEs) in environmental and human samples. Concentrations of PBDEs in herring gull eggs, seal blubber, and human breast milk are increasing at an almost exponential rate with doubling times ranging from three to nine years [1–3]. Levels of PBDEs in North American human breast milk are almost two orders of magnitude higher than levels measured in Europe [4]. This trend is most likely due to the heavier use of PBDEs in North America relative to Europe as these compounds have been recently phased out in the European Union [5].

Polychlorinated biphenyls (PCBs) were banned from use and production in the United States in the 1970s because of their persistence and toxicity to humans and wildlife. Currently, PBDEs are displaying a fate similar to PCBs in their persistence and ubiquity throughout the world. The recent detection of PBDEs in the Canadian Arctic and in Antarctica [2,6] demonstrates their potential for long-range transport, similar to PCBs. Physicochemical properties such as vapor pressures and Kow of these two classes of compounds are comparable, which would suggest similar behavior in the environment. A limited number of toxicity studies conducted to date indicate that some BDE congeners have the potential to elicit adverse health effects in exposed organisms. For example, PBDEs have been shown to adversely affect thyroid hormone homeostasis and neurobehavioral development [7,8]. Some field studies indicate that levels of total PBDEs in fish tissues are currently comparable to, and in some areas greater than, levels of total PCBs [9,10].

Most environmental samples quantified for PBDEs involve measurement of congeners primarily found in one of the commercial formulations known as pentaBDE. This product consists predominantly of five congeners, BDE 47 (2,2′,4,4′-tetrabromodiphenyl ether), BDE 99 (2,2′,4,4′,5-pentabromodiphenyl ether), BDE 100 (2,2′,4,4′,6-pentabromodiphenyl ether), BDE 153 (2,2′,4,4′,5,5′-hexabromodiphenyl ether), and BDE 154 (2,2′,4,4′,5,6-hexabromodiphenyl ether), in the ratio 33, 49, 9, 5, and 4% of the total composition, respectively. This composition is strikingly similar to the composition observed in many biota samples collected from around the world and implicates its use as a source to these food webs [9–16].

A few field studies have examined the concentration and congener distribution of BDEs in the common carp (Cyprinus carpio) [9,10,12,17]. An intriguing pattern of BDE accumulation was observed in all these studies. Unlike most other biota samples in which BDE 99 makes up 10 to 60% of the total BDE burden, the BDE 99 burden in carp is <1% and in many cases is at concentrations below detection. Only three explanations exist for the lack of BDE 99 in carp tissues: Either carp were not exposed to BDE 99 in the field, BDE 99 is not bioavailable for accumulation in fish tissues, or carp efficiently
metabolize BDE 99. Since BDE 99 has been identified in the tissues of other fish species collected in the field and laboratory exposures have shown BDE 99 to be bioavailable to pike tissues (Esox lucius) [18], the lack of BDE 99 in exposed carp likely results from metabolic processes.

The BDE metabolism data in fish is restricted to a few species [19]. In one study, a single dose of BDE 47 was administered to captive northern pike (Esox lucius) via a natural food matrix of brown trout (Salmo trutta) [20]. Selective retention of six hydroxylated (HO)-BDE metabolites was observed in a number of tissues, including plasma and liver (e.g., 2-HO-2',4,4',6-tetraBDE and 2-HO-2',3,4,4'-tetraBDE, which were characterized by comparison with synthesized standards). In pike, BDE 47 was not readily metabolized into metabolites that could be easily eliminated since the concentration in lipid-rich tissues did not diminish significantly with time.

While numerous laboratory studies have investigated the bioaccumulation and fate of PCBs within aquatic organisms [21–25], very few laboratory studies have explored the bioaccumulation potential of BDE congeners. To our knowledge, only two laboratory studies have been reported in which organisms were exposed to BDE congeners commonly found in the pentaBDE formulation [18,26]. The present study was designed to examine the fate of BDE congeners in carp tissues under controlled laboratory conditions. The results will provide additional information on BDE exposure in fish tissues. The objectives of this investigation were to assess the accumulation potential of individual BDE congeners to carp tissues, examine the ability of carp to metabolize BDEs, and compare the accumulation behavior of BDEs versus PCBs.

**EXPERIMENTAL METHODS**

**Tank design**

The laboratory experiment was designed as a repeated-measures study in which we used one control tank and three replicate exposure tanks to monitor congener assimilation. Approximately 50 juvenile carp, about 100 mm in length, were purchased from Hunting Creek Fisheries in Thurmont (MD, USA) and transferred to the Chesapeake Biological Laboratory in Solomons (MD, USA). Twelve fish were randomly assigned to each of four 132-L round polyethylene tanks. Filtered well water was heated in a head tank to maintain a constant temperature of 22°C and delivered to each of the individual tanks to supply fresh water. The flow rate of the incoming water was approximately 1.0 L/min, and a stand pipe was placed in the middle of each tank for water drainage. Under these conditions, water in the tanks had a residence time of approximately 2 h. Air stones were placed in each tank to maintain oxygen saturation in the water, and temperature and flow rates were monitored throughout the duration of the experiment. Fish were fed clean control food for a week to acclimatize them to their new surroundings prior to beginning the experiment.

**Food exposure**

Carp exposure to PCB and BDE congeners was accomplished via the consumption of contaminant-spiked food. To create the food mixture, frozen bloodworms (San Francisco Bay Brand, Newark, CA, USA) were homogenized in a glass blender and mixed with 20% (by mass) fish food pellets purchased from Hunting Creek Fisheries. The BDE congeners 28 (2,4,4'-tribromodiphenyl ether), BDE 47, BDE 99, BDE 153, and PCB congeners 52 (2,2',5,5'-tetrachlorobiphenyl), 153 (2,2',4,4',5,5'-hexachlorobiphenyl), and 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) were purchased from Cambridge Isotopes Laboratories (Andover, MA, USA), weighed out, and dissolved in 20 ml cod liver oil. The oil solution was thoroughly blended with the bloodworm mixture and then stored in plastic bags in the freezer until use. Pieces of this frozen mixture were broken off into pieces to feed to the fish. Food was rapidly consumed within 30 s after addition to the tanks, and very little disintegration of the food was observed. The fish food cocktail containing PCBs and BDEs was spiked in an attempt to reach a concentration of approximately 100 ng/g wet weight of each congener. Fish were exposed to approximately 100 ng of each congener per day, with the exception of BDE 99, where exposure was greater, 470 ± 38 ng/d, because this was the congener of most interest with regard to bioaccumulation potential. This exposure concentration is higher than most reported concentrations of BDEs in biota collected in the wild [27]; however, this exposure is also significantly lower than concentrations shown to elicit toxic effects [28–30]. Control food was prepared by homogenizing bloodworms with 20% (by mass) fish food pellets and spiking with 20 ml pure cod liver oil. Fish were fed 1 g/d/fish of either the spiked food pellets or the control food, depending on the tank, for 60 d. The pellets sank to the bottom of the tank and were rapidly consumed by the fish in the first 30 s. After 60 d of exposure, all the remaining fish in the tanks were fed control food for a further 40 d to monitor depuration.

**Sampling**

One fish from each tank was sampled on days 0, 5, 10, 20, 30, 45, 60, 69, 85, and 100. Fish mass and length were recorded, and fish were killed by cervical dislocation. Blood samples were taken from the dorsal aorta using heparanized syringes and pooled among fish from replicate treatment tanks. Blood samples were centrifuged at 3,000 rpm for 20 min, and the serum was collected for analyses of potential phenolic PCB and BDE metabolites. The stomach cavity from each fish was removed of its contents, the liver removed, and its mass recorded. Livers from replicate exposures were combined for the analyses of parent exposure compounds. The remaining carcasses were then homogenized in minchoppers and stored in precleaned glass jars and stored at −20°C until analysis.

Whole-body and pooled liver samples were extracted for PCB and BDE analysis using Soxhlet extraction. Fish tissues (~5 g for whole-body tissues) were first ground with Na2SO4, spiked with two surrogate standards (2,4,4’,6-tetrabromodiphenyl ether, BDE 75, and 2,3,4,4’,5,6-hexachlorobiphenyl, PCB 166) and extracted with dichloromethane in Soxhlets for approximately 24 h. Lipid content was determined on the extracts using gravimetric analysis. Extracts were concentrated using rotoevaporation, followed by bulk lipid removal using gel permeation chromatography and final cleanup by Florisil (U.S. Silica, Berkeley Springs, WV, USA) chromatography. An internal standard of 13C-labeled 2,2’,3,4,4’,5,6-heptabromodiphenyl ether, BDE 183, was added to each sample for quantification of BDEs, while 2,2’,3,4,4’,5,6,6’-octachlorobiphenyl, PCB 204, was added to quantify PCBs. Recovery of the surrogate standards averaged 81 ± 5% and 89 ± 9% for BDE 75 and PCB 166, respectively. The Na2SO4 blanks were extracted and quantified for BDEs and PCBs alongside the fish extracts. Some minor laboratory contamination occurred for BDEs 47 and 99 (<0.5 ng), but levels were sufficiently low to exclude blank correction procedures. Method detection lim-
its (MDLs) were calculated by taking the lowest possible area for a peak (just above the noise) and determining the maximum mass of each congener that area would represent. Thus, the MDLs for BDEs were 0.57 ± 0.47, 1.04 ± 1.15, 2.0 ± 2.21, and 1.48 ± 0.43 ng for BDE 28, 47, 99, and 153, respectively. Both MDLs for PCBs were 0.67 ± 0.17, 1.24 ± 0.32, and 0.84 ± 0.22 ng for PCBs 52, 153, and 180, respectively. Analytical precision (repeated injections of one sample five times) for our sample quantification averaged 8 ± 5%.

Single serum samples from days 45, 60, 69, and 85 of the exposure and depuration phases were analyzed at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, ON Canada) for potential HO-BDEs according to the published methods [31,32] with some modifications. Briefly, each thawed serum sample was transferred to a 50-ml screw-top centrifuge tube and accurately weighed. Each sample was spiked with the internal standards 4′-HO-2,2′,4′-tribromodiphenyl ether (4′-HO-BDE 17, 50 ± 46.8 pg/µl) and 4-HO-2,3′,5,5′-tetrachlorobiphenyl (4-HO-PCB 72; 100 µl of 1114.4 pg/µl), followed by acidification with HCl (6 M, 1 ml) and addition of 2-propanol (3 ml). The denatured plasma was extracted three times with an equal volume of methyl-tert-butyl ether (MtBE):hexane (1:1). The organic extracts were partitioned with potassium hydroxide (1 M in 50% ethanol). The alkaline phase was acidified, and the phenolic compounds were reextracted in hexane: MtBE (1:1), dried over sodium sulfate, and derivatized to their methoxy-analogues via a methylation reaction using diazomethane. The methoxy-analogues were purified on a silica/sulfuric acid (22%) column fraction were compared with the MeO-BDE (1.0 ng/ml each) standard mixture of MeO-BDEs (10 congeners). These ions were subsequently used for the analyte determinations in the samples. Quantification ions were [M]+ or [M + 2Br]+, and identify confirmation ions were [M + 2]+ or [M + 2 – 2Br]+, which reflect the 41Br and 85Br isotopic contributions. The ECD chromatograms and HRMS chromatograms of the phenolic fraction were compared with the MeO-BDE (1.0 ng/ml each) external standard mixture. The MeO-BDE standards were generously synthesized and supplied by G. Marsh and A. Bergman (Stockholm University, Stockholm, Sweden) [34].

Data analysis

Depuration of PCB and BDE congeners was calculated using a first-order rate loss equation:

\[ C_t = C_0 \times \exp(-kt) \]  

where \( C \) equals concentration, \( k \) is the depuration rate, and \( t \) is time in days. Rearrangement of Equation 1 allows one to plot \( \ln [C_t]/[C_0] \) versus time and determine the slope \( k \). The net assimilation efficiency of each PCB and BDE congener was calculated using the following equation:

\[ \alpha = \frac{\text{body burden in fish at time } t}{\text{cumulative mass exposed to fish up to time } t} \]  

where \( \alpha \) is net assimilation efficiency. It was very difficult to calculate the direct assimilation efficiency of these congeners, as this was an aquatic exposure. Most assimilation efficiency calculations involve measurements of ingestion minus egestion and excretion. Here we have explored net assimilation efficiency given that our goals were primarily to examine the differences in assimilation and depuration between BDE and PCB congeners. Although the absolute value of the efficiency may vary in different environments and conditions, the relative efficiencies of the PCBs and BDEs should remain similar. This calculation allowed us to compare the assimilation efficiencies of PCBs with BDEs.

Biomagnification was determined in our study using the following equation:

\[ \text{BMF} = \frac{(U/F)}{k} \]
Table 1. Growth parameters (mean ± standard deviation; n = 3) during the exposure of juvenile carp to polychlorinated biphenyls and polybrominated diphenyl ethers

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Mortality</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Growth rate (10⁻³/d)*</td>
<td>7.7 ± 0.1</td>
<td>8.7 ± 0.3</td>
</tr>
<tr>
<td>Fish mass, day 0</td>
<td>17.0 ± 0.3</td>
<td>18.1 ± 3.1</td>
</tr>
<tr>
<td>Fish mass, day 100</td>
<td>48.2 ± 0.2</td>
<td>43.1 ± 7.6</td>
</tr>
<tr>
<td>% Lipid, day 30</td>
<td>2.91 ± 1.2</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>% Lipid, day 60</td>
<td>3.6 ± 0.1</td>
<td>5.9 ± 1.3</td>
</tr>
<tr>
<td>% Lipid, day 100</td>
<td>5.6 ± 0.4</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td>Liver somatic indexb</td>
<td>2.16 ± 0.3</td>
<td>2.21 ± 0.6</td>
</tr>
</tbody>
</table>

* Growth rate was calculated using ln weight = a + b(time), where b = growth rate.

b Liver somatic index = mass liver/mass whole body × 100%.

where \( U \) is the net rate of assimilation (ng/g/d; calculated from the slope of concentration versus time), \( F \) is the concentration in the food (ng/g), and \( k \) is the depuration rate (per day). All statistical analyses were conducted using the mixed procedure model in the software package SAS® (Ver 8.0, Cary, NC, USA) to test for significant differences among exposure treatments, replicate tanks, and time.

RESULTS AND DISCUSSION

Growth

During the 100-d experiment, no mortality in either the control group or the exposure group was observed. No significant differences were seen in the growth rates of fish between the control and the exposed tanks or among replicate tanks (\( F_{1,3} = 4.12, p = 0.14 \)). Carp increased their body lipid content from approximately 2 to 6% between days 1 and 100, respectively (Table 1), and again no significant differences were seen in lipid between the control and exposure treatments (\( p < 0.05 \)). Liver somatic index (mass of liver/body mass) was also monitored throughout the duration of the experiment and did not change significantly during the study or between groups.

Exposure

The exposure rate for each BDE and PCB congener is listed in Table 2. Control fish were measured for the concentration of BDE and PCB congeners in their tissues due to laboratory or background contamination. Low concentration (<3.0 ng/g) residues of PCBs 153 and 180 and BDEs 47 and 99 were detected in control fish throughout the exposure. However, all concentrations of PCBs and BDEs were significantly different (\( p < 0.05 \)) than control fish concentrations by day 20.

On day 5, five of the seven PCB and BDE congeners were detected in both liver and whole-body tissues. The BDE 153 was detected in the body tissue beginning on day 10, while BDE 99 was not detected in body tissue at all throughout the experiment despite the fourfold higher concentration of BDE 99 in the food. Concentrations of both PCBs and BDEs continued to increase in exposed fish throughout the 60-d exposure.

Accumulation in body tissues

Net assimilation rates for whole-body tissues are displayed in Table 2. Concentrations of all congeners (with the exception of BDE 99) increased linearly over the exposure period. Linear regressions of the net assimilation versus time displayed \( r^2 \) values of 0.9212, 0.9451, and 0.9301 for PCB congeners 52, 153, and 180, respectively, whereas the \( r^2 \) values for BDE congeners 28, 47, and 153 were 0.8073, 0.8037, and 0.8579, respectively. Values are not reported here on a lipid basis because normalization to lipid increased the variance in assimilation rates.

Polychlorinated biphenyl net assimilation in whole-body tissues ranged from 0.61 ± 0.13 to 1.1 ± 0.31 ng/g/d for all three PCB congeners and was comparable to the assimilation of BDE 28. On day 60, all three PCB congeners displayed comparable concentrations in fish tissues (Fig. 1). However, the four BDE congeners displayed much different concentrations on day 60, and in fact the absolute concentrations spanned three orders of magnitude (Fig. 1B). Rapid assimilation of BDE 47 was observed during the exposure period and was greater relative to the assimilation of all other analytes (\( p < 0.05 \)). As mentioned earlier, little assimilation of BDE 153 was observed (0.16 ng/g/d), and no accumulation of BDE 99 occurred. Distinct differences exist in the assimilation rates and accumulation behavior of these PCB and BDE congeners despite their comparable exposure rates and similar physicochemical properties. If passive diffusion were the only factor influencing the assimilation of these congeners in carp tissues, we would expect similar rates of accumulation. Our results suggest that either different processes are controlling the assimilation of PCBs relative to BDE congeners into carp tissues.

Table 2. Polybrominated diphenyl ether (BDE) and polychlorinated biphenyl (PCB) congener–specific exposure, assimilation, and depuration parameters calculated from laboratory results

<table>
<thead>
<tr>
<th>Congener</th>
<th>Homologue group</th>
<th>Food exposure (ng/d)</th>
<th>Net assimilation (ng/g/d)</th>
<th>Liver assimilation (ng/g/d)</th>
<th>Depuration rate (10⁻³/d)</th>
<th>Half-life (d)</th>
<th>BMF*</th>
<th>Net assimilation efficiency (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 52</td>
<td>Tetra</td>
<td>58 ± 5</td>
<td>0.61 ± 0.13</td>
<td>1.15</td>
<td>1.5 ± 0.2</td>
<td>46.2 ± 6</td>
<td>0.701</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>PCB 153</td>
<td>Hexa</td>
<td>100 ± 8</td>
<td>1.11 ± 0.31</td>
<td>1.68</td>
<td>1.5 ± 0.2</td>
<td>46.2 ± 3</td>
<td>0.740</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>PCB 180</td>
<td>Hepta</td>
<td>95 ± 5</td>
<td>0.99 ± 0.29</td>
<td>1.45</td>
<td>2.1 ± 0.1</td>
<td>33.0 ± 2</td>
<td>0.496</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>BDE 28</td>
<td>Tri</td>
<td>96 ± 3</td>
<td>0.65 ± 0.13</td>
<td>1.23</td>
<td>1.9 ± 0.9</td>
<td>36.5 ± 17</td>
<td>0.356</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>BDE 47</td>
<td>Tetra</td>
<td>174 ± 7</td>
<td>5.44 ± 1.63</td>
<td>7.33</td>
<td>2.3 ± 0.9</td>
<td>30.1 ± 12</td>
<td>1.359</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>BDE 99</td>
<td>Penta</td>
<td>470 ± 38</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>BDE 153</td>
<td>Hexa</td>
<td>112 ± 7</td>
<td>0.16 ± 0.06</td>
<td>0.47</td>
<td>5.1 ± 3.6</td>
<td>13.6 ± 9</td>
<td>0.028</td>
<td>4 ± 3</td>
</tr>
</tbody>
</table>

* BMF = biomagnification factor (ratio of normalized assimilation rate to depuration rate).

b Assimilation efficiency (body burden, i.e., concentration of congener-mass of fish) in fish normalized to cumulative exposure during 60-d exposure period.

NA = not applicable.
Accumulation in liver tissues

Accumulation of BDEs and PCBs in the liver followed trends similar to whole-body tissues. Absolute concentrations of all analytes in liver tissues were 1.1 to 7.7 times higher than those in whole-body tissues on both a wet-weight and a lipid-weight basis. Relative assimilation rates for each congener were also greater in liver tissues compared to whole-body tissues. Temporal trends in blood serum over the 40-d period were very similar to those observed in whole-fish tissues. On day 60, the measured levels were 5.2, 45.1, 0.1, and 1.2 ng/g wet weight for BDE congeners 28, 47, 99, and 153, respectively. The PCB concentrations in blood serum on day 60 were 10.3, 7.0, and 12.0 ng/g wet weight for PCB congeners 52, 153, and 180, respectively. Partitioning between blood serum and whole-body tissues were similar for both PCBs and BDEs with the exception of PCB 52, which displayed a higher ratio. No temporal trends in the partitioning of congeners between blood and whole-body tissues were observed over the sampling period. Figure 2 displays the average ratio of each congener in blood serum for the four sampling time points.

Depuration

Polychlorinated biphenyls displayed depuration rates ranging from 0.015 to 0.021/d and our results compare very well to similar dosing studies with juvenile rainbow trout [24]. Depuration rates for BDEs were highly variable among replicates compared to PCBs (Table 2), and thus no significant difference was seen in depuration between rates for PCBs and BDEs in the present study.

Depuration half-lives of PCBs in carp tissues were as low as 33 d for PCB 180 and as high as 46 d for PCB 52 and PCB 153. Previous studies on BDE bioaccumulation have shown significant accumulation of BDE 99 in laboratory-exposed blue mussels and pike [18,26] and field-collected fish [19,10,12]. Previous studies on BDE bioaccumulation have shown significant accumulation of BDE 99 in laboratory-exposed blue mussels and pike [18,26] and field-collected fish [19,10,12]. Precious studies on BDE bioaccumulation have shown significant accumulation of BDE 99 in laboratory-exposed blue mussels and pike [18,26] and field-collected fish [19,10,12].

The liver aids in the metabolism and breakdown of foreign substances in complex organisms, and the endogenous enzymes aid in the breakdown of PCBs to hydroxyl- and methyl-sulfonyl derivatives [36]. The placement and orientation of the liver in carp is different relative to other fish species in that it is not a compact organ anterior to the intestinal mass. The liver in carp extends longitudinally and wraps around the intestinal mass throughout the entire length of the gastrointestinal tract. This physical contact acts to increase the surface area contact with the intestine and could increase the capacity for metabolism of contaminants absorbed through dietary intake.

**HO-BDEs and HO-PCBs in carp serum**

Both HO-PCBs and HO-BDEs are chemical markers for the potential metabolic capacity of the CYP enzyme system to biotransform PCBs and BDEs, respectively. The HO-BDE formation indicates one possible pathway for elimination, but other pathways are possible, including debromination and non-metabolic diffusion, such as passive diffusion via the gill and GI tract [19]. Despite consistent recovery of the HO-BDE and HO-PCB internal standards, neither HO-PCB nor HO-BDE metabolites were detectable—<1 pg/g wet wt by either ECD or HRMS(EI)—in the serum of the exposed carp. The lack of detectable HO-BDE metabolites suggests several possibilities in relation to BDE metabolism and metabolite retention processes. It is possible that HO-BDEs are formed in carp via CYP enzymes and subsequent epoxide hydrolase mediation; however, phase II conjugating processing may be effective at removing HO-BDEs at a similar rate as they are formed [19]. Another possibility is that HO-BDEs have low affinity for binding proteins in the blood of carp. However, this is unlikely since a number of halogenated phenolic contaminants, including HO-PCBs and HO-BDEs, have been reported in the blood plasma of wild carp from the Detroit River (Detroit, MI, USA) [32,37]. Additionally, it is possible that carp do not have the capacity to produce HO-BDEs and that previous measurements of HO-BDEs in field-collected carp tissues are a result of accumulation from their environment.

Despite the lack of HO-BDEs and HO-PCBs in the blood serum, the parent BDE and PCB congeners were detected and measured in the blood serum. Pooled serum was measured for BDE and PCB congeners on days 45, 60, 69, and 85. Temporal trends in blood serum over the 40-d period were very similar to those observed in whole-fish tissues. On day 60, the measured levels were 5.2, 45.1, 0.1, and 1.2 ng/g wet weight for BDE congeners 28, 47, 99, and 153, respectively. The PCB concentrations in blood serum on day 60 were 10.3, 7.0, and 12.0 ng/g wet weight for PCB congeners 52, 153, and 180, respectively. Partitioning between blood serum and whole-body tissues were similar for both PCBs and BDEs, excepting the exception of PCB 52, which displayed a higher ratio. No temporal trends in the partitioning of congeners between blood and whole-body tissues were observed over the sampling period. Figure 2 displays the average ratio of each congener in blood serum and whole-body tissues for the four sampling time points.

**Accumulation in liver tissues**

Accumulation of BDEs and PCBs in the liver followed trends similar to whole-body tissues. Absolute concentrations of all analytes in liver tissues were 1.1 to 7.7 times higher than those in whole-body tissues on both a wet-weight and a lipid-weight basis. Relative assimilation rates for each congener were also greater in liver tissues compared to whole-body tissues (see Table 2).

The liver aids in the metabolism and breakdown of foreign substances in complex organisms, and the endogenous enzymes aid in the breakdown of PCBs to hydroxyl- and methyl-sulfonyl derivatives [36]. The placement and orientation of the liver in carp is different relative to other fish species in that it is not a compact organ anterior to the intestinal mass. The liver in carp extends longitudinally and wraps around the intestinal mass throughout the entire length of the gastrointestinal tract. This physical contact acts to increase the surface area contact with the intestine and could increase the capacity for metabolism of contaminants absorbed through dietary intake.

**HO-BDEs and HO-PCBs in carp serum**

Both HO-PCBs and HO-BDEs are chemical markers for the potential metabolic capacity of the CYP enzyme system to biotransform PCBs and BDEs, respectively. The HO-BDE formation indicates one possible pathway for elimination, but other pathways are possible, including debromination and non-metabolic diffusion, such as passive diffusion via the gill and GI tract [19]. Despite consistent recovery of the HO-BDE and HO-PCB internal standards, neither HO-PCB nor HO-BDE metabolites were detectable—<1 pg/g wet wt by either ECD or HRMS(EI)—in the serum of the exposed carp. The lack of detectable HO-BDE metabolites suggests several possibilities in relation to BDE metabolism and metabolite retention processes. It is possible that HO-BDEs are formed in carp via CYP enzymes and subsequent epoxide hydrolase mediation; however, phase II conjugating processing may be effective at removing HO-BDEs at a similar rate as they are formed [19]. Another possibility is that HO-BDEs have low affinity for binding proteins in the blood of carp. However, this is unlikely since a number of halogenated phenolic contaminants, including HO-PCBs and HO-BDEs, have been reported in the blood plasma of wild carp from the Detroit River (Detroit, MI, USA) [32,37]. Additionally, it is possible that carp do not have the capacity to produce HO-BDEs and that previous measurements of HO-BDEs in field-collected carp tissues are a result of accumulation from their environment.

Despite the lack of HO-BDEs and HO-PCBs in the blood serum, the parent BDE and PCB congeners were detected and measured in the blood serum. Pooled serum was measured for BDE and PCB congeners on days 45, 60, 69, and 85. Temporal trends in blood serum over the 40-d period were very similar to those observed in whole-fish tissues. On day 60, the measured levels were 5.2, 45.1, 0.1, and 1.2 ng/g wet weight for BDE congeners 28, 47, 99, and 153, respectively. The PCB concentrations in blood serum on day 60 were 10.3, 7.0, and 12.0 ng/g wet weight for PCB congeners 52, 153, and 180, respectively. Partitioning between blood serum and whole-body tissues were similar for both PCBs and BDEs, excepting the exception of PCB 52, which displayed a higher ratio. No temporal trends in the partitioning of congeners between blood and whole-body tissues were observed over the sampling period. Figure 2 displays the average ratio of each congener in blood serum and whole-body tissues for the four sampling time points.

**Depuration**

Polychlorinated biphenyls displayed depuration rates ranging from 0.015 to 0.021/d and our results compare very well to similar dosing studies with juvenile rainbow trout [24]. Depuration rates for BDEs were highly variable among replicates compared to PCBs (Table 2), and thus no significant difference was seen in depuration between rates for PCBs and BDEs in the present study.

Depuration half-lives of PCBs in carp tissues were as low as 33 d for PCB 180 and as high as 46 d for PCB 52 and PCB 153. Previous studies on BDE bioaccumulation have shown significant accumulation of BDE 99 in laboratory-exposed blue mussels and pike [18,26] and field-collected fish [19,10,12]. Previous studies on BDE bioaccumulation have shown significant accumulation of BDE 99 in laboratory-exposed blue mussels and pike [18,26] and field-collected fish [19,10,12]. Previous studies on BDE bioaccumulation have shown significant accumulation of BDE 99 in laboratory-exposed blue mussels and pike [18,26] and field-collected fish [19,10,12].
The average half-lives of BDEs ranged from 14 d for BDE 153 to 37 d for BDE 28. In a study by Gustafsson et al. [26], blue mussels were exposed to both PCBs and BDEs from spiked algae. The depuration rate of BDE 47 was three times higher than the depuration of PCB 153, and thus PCB 153 had a longer half-life in mussel tissues. In contrast, BDE 47 and PCB 153 depuration rates were comparable in the present study. Therefore, the relative fate of PCBs and BDEs appears to be different in different types of organisms, perhaps because of species-specific biotransformation capacities.

In previous fish studies, an inverse relationship between depuration rate and log $K_{ow}$ has been observed [25]. In general, bioaccumulation tends to increase up to a log $K_{ow}$ value of 7 and then level off [38]. Molecules with higher log $K_{ow}$ values will have a stronger affinity to lipidlike biomolecules and thus increase their lifetimes in tissues. Within the 1.5-order-of-magnitude difference in $K_{ow}$ values for PCBs in this study, no consistent trend was observed in the relationship between half-lives and log $K_{ow}$ (Fig. 3). However, a decrease in half-lives of BDE congeners was observed with increasing log $K_{ow}$, contrary to trends seen with PCBs in other studies. The low half-lives of BDEs suggest that alternative elimination routes (metabolism) are affecting the lifetimes of BDEs in carp tissues.

**Net assimilation efficiency and biomagnification**

In addition to examining the fate of these compounds in fish tissues, it is also important to examine the efficiency at which the compound is assimilated into the exposed fish. In most laboratory experiments, this can be easily accomplished by quantifying the input and output mass of the chemical ingested and egested from the organism. However, with aquatic organisms, this process can be somewhat difficult. In our study, we quantified the net efficiency at which each congener was accumulated by normalizing the body burden of each congener to their cumulative exposure (Eqn. 2). The percentage of the cumulative exposure accumulated by carp throughout the exposure is presented in Figure 4. Approximately 40% of the PCB exposure accumulated in carp after 60 d, which compares well with the results reported by Fisk et al. [25] for juvenile rainbow trout, by Gobas et al. [22] for goldfish, and by Sijm et al. [39] for guppies, but are lower than values observed in rainbow trout and pike [18,21].

The BDEs displayed significantly different net assimilation efficiencies among each congener (tested using standard analysis of variance techniques). Only about 5% of BDE 153 and
Dietary accumulation of PBDEs and PCBs by juvenile carp

Table 3. A comparison of polybrominated diphenyl ether (BDE) and polychlorinated biphenyl (PCB) net assimilation efficiencies between common carp (Cyprinus carpio) and northern pike (Esox lucius)

<table>
<thead>
<tr>
<th>Congener</th>
<th>Common carp (this study)</th>
<th>Northern pike (Bureau et al. [18])</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE 28</td>
<td>20 ± 7</td>
<td>NM*</td>
</tr>
<tr>
<td>BDE 47</td>
<td>93 ± 10</td>
<td>90 ± 20</td>
</tr>
<tr>
<td>BDE 99</td>
<td>0</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>BDE 153</td>
<td>4 ± 3</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>PCB 52</td>
<td>38 ± 7</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>PCB 153</td>
<td>43 ± 4</td>
<td>70 ± 10</td>
</tr>
<tr>
<td>PCB 180</td>
<td>38 ± 3</td>
<td>NM</td>
</tr>
</tbody>
</table>

*a NM = not measured.

20% of BDE 28 was assimilated by carp, while almost 100% of BDE 47 was assimilated. In a study by Bureau et al. [18] in which pike where exposed through the diet, BDE 47 displayed an uptake efficiency comparable to PCB 153 that the authors attributed to similarities in their log Kow values. As seen in Figure 3B, no trend was observed between net assimilation efficiency and log Kow. The net assimilation of BDE 47 was two times greater than the assimilation of PCB 153 despite their similar log Kow values. Table 3 presents a comparison of net assimilation efficiencies of common carp (this study) with the uptake efficiencies of northern pike calculated by Bureau et al. [18]. Pike appear to have a greater assimilation efficiency for BDE congeners, as approximately 60% of the BDE 99 and 40% of the BDE 153 exposure was taken up into pike tissues, which is in sharp contrast to the assimilation efficiencies of these congeners in carp.

The net assimilation efficiency of BDE 47 in our study is very high. In fact, all congeners except BDE 47 reached a steady-state net assimilation efficiency by day 20, but the assimilation of BDE 47 continued to increase during the 60-d exposure period from 40% on day 20 to approximately 93% at the end of the experiment. This increase in net assimilation efficiency over time indicates either that carp increase their capacity to assimilate this congener or perhaps that another source of BDE 47 was introduced. It seems improbable to conclude that the ability of carp to assimilate BDE 47 would change so significantly. In Figure 4, we have presented the cumulative mass of each congener accumulated in fish tissues on discrete time points throughout the experiment. On day 20 of the experiment, less than 50% of the BDE 47 exposure had been assimilated in the tissues of the exposed carp (more than half the BDE 47 ingested was not assimilated during the first 20 d). However, by day 60, the body burden in carp is comparable to 100% of the entire exposure mass from all 60 d of feeding, which is impossible since assimilation was less than 50% earlier in the experiment. This leads us to conclude that another source of BDE 47 was introduced to the carp during the experiment.

The fact that BDE 99 did not accumulate in carp tissues is intriguing since other studies have identified BDE 99 in fish tissues and laboratory exposures have shown that other organisms accumulate this compound. The BDE 99 is a pentabrominated compound with a 2,2’,4,4’,5-substitution pattern, while BDE 47 is a tetra-brominated compound with a 2,2’,4,4’-substitution pattern. The removal of a bromine atom from the meta-position of the phenyl ring on BDE 99 results in the formation of BDE 47. It is possible that biotransformation of some of the BDE 99 may lead to the formation of BDE 47, which could be accumulated in carp tissues. Therefore, BDE 99 may not accumulate in carp tissues because it is readily metabolized and/or degraded within the gut to other products, including BDE 47. Bureau et al. [20] studied the fate of radiolabeled BDE 47 in pike and found that the distribution of the labeled BDE 47 in the fish tissues suggested the formation of hydrophobic metabolites of BDE 47 rather than hydrophilic metabolites. They hypothesized that reductional dehalogenation occurring in the liver via mediation by the cytochrome P450 system produced debrominated products. A detailed study of the assimilation and debromination of BDE 99 and BDE 209 has been investigated and supports the formation of BDE 47 from debromination of BDE 99 in the gut [40,41].

Biomagnification factors ranged from 0.028 for BDE 153 to 1.36 for BDE 47. The bioaccumulation factor of BDE 47 is significantly higher than the values for PCBs and suggests that it may reach levels of concern in fish exposed to moderate levels in the environment. In a study by Gustafsson et al. [26] in which blue mussels were exposed to PCBs and BDEs, the biomagnification of BDE 47 was six times greater than the biomagnification of PCB 153. In our study, we have also observed a greater biomagnification for BDE 47 relative to PCB 153, although only about two times greater.

**Implications**

In a previous field study [16], we observed similar bioaccumulation of BDE 47 and PCB 153 among zooplankton, alewife, and lake trout, suggesting that these two types of compounds possess similar bioaccumulation potentials in food webs. However, it now seems clear that some species, specifically the common carp, differ in their capacity for assimilating these two types of contaminants. The low net assimilation efficiencies of BDE 28 and BDE 153 (20 and 4%, respectively) and high depuration rates suggest that they have higher bio-transformation capacities and/or lower assimilation potential relative to PCBs of similar Kow. Based on our results, it remains unclear whether BDE 99 is assimilated and rapidly debrominated or is debrominated in the gut by microorganisms leading to assimilation of BDE 47. Further work is needed to identify the mechanism(s) responsible for debromination of PBDEs and to measure the rates at which they occur.

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