Bioavailability and mass balance studies of a commercial pentabromodiphenyl ether mixture in male Sprague–Dawley rats

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Abstract

Polybrominated diphenyl ethers (PBDE) are common flame retardants used in polyurethane foam, high impact polystyrene, and textiles which appear to be increasing in the environment and biota. Two PBDE congeners that are particularly prominent in environmental samples are 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99). These two congeners are major components in penta-BDE formulations which constitute a minor percentage of the commercial PBDE market. In order to determine the bioavailability and bioconcentration potential of these PBDEs, we have conducted a feeding experiment in rats, dosing with low amounts of a commercial penta-BDE mixture for 21 days to mimic an environmental exposure. The carcasses, livers, and feces from control and dosed rats were quantitated for PBDEs by a high resolution GC–MS isotope dilution method. Between 25% and 50% of each of the dosed congeners was retained in the rats with the liver being a minor depot (<1% of the dose). Fecal excretion accounted for 4–12% of the dosed congeners. A large percent of the dose (40–60%) was not recovered indicating that metabolic transformations may have occurred in the rats. Hydroxylated metabolites were qualitatively identified in the feces and carcass by GC–MS. The relative congener distribution in each tissue was nearly identical to the congener distribution of the commercial mixture. Conclusions from the study suggest that the tetra- to hexa-BDEs present in commercial penta-BDE formulations are largely bioavailable, that bioavailability in the rat is not dependent on the degree of bromination, and that metabolism may occur to a large extent during a chronic exposure.

Keywords: PBDEs; Bioconcentration; Accumulation; Metabolites; DE-71

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are common additive flame retardants manufactured as three different formulations for use in commercial products. Penta-BDE formulations are used mainly in textiles and polyurethane foams including carpet, furniture, and sound insulation panels; octa-BDE formulations are used mainly in acrylonitrile butadiene styrene (ABS) resins for computer and appliance housings; and deca-BDE formulations are used in numerous resins and polymers including high-impact polystyrene for computers, televisions, and other electronic equipment (World Health Organization, 1994). Recent production figures indicate that the global demand for these materials increased from 40000 Mt ons in 1992 (World Health Organization, 1994) to over 67000 Mt ons in 1999 where it has remained steady through 2003 (www.bsef.com1). PBDEs have some structural similarity to other environmentally persistent contaminants, i.e., polychlorinated dioxins, polychlorinated furans, and polychlorinated biphenyls, making them suspect chemical hazards. Moreover, evidence of adverse health effects in animals due to PBDEs continues to grow (Darnerud, 2003). Together, the increase in production and the elucidation of toxic effects have made PBDEs an environmental contaminant of foremost concern. Recently, the production and use of penta- and octa-BDE formulations have

1 Bromine Science and Environmental Forum website.
been banned or restricted in Europe and the USA, but products containing these formulations and environmental reservoirs remain.

Environmental sampling for the past 20 years has shown that PBDEs are persistent in sediment and bioaccumulate in tissues (Watenabe et al., 1987; Haglund et al., 1997; de Wit, 2002). Levels in human milk and serum appear to be increasing (Norén and Meironytė, 2000; Ryan et al., 2002; Sjödin et al., 2004) as are levels in organisms that inhabit the oceans (de Boer et al., 1998; de Wit, 2002). The major PBDEs reported in fish and fish-eating mammals are 2,2′,4,4′-tetrabrominated (BDE-47) followed by 2,2′, 4,4′,5-penta-BDE (BDE-99), 2,2′,4,4′,6-penta-BDE (BDE-100), 2,2′,4,4′,5,5′-hexabrominated (BDE-153), and 2,2′,4,4′,5,6′-hexabrominated (BDE-154) (Haglund et al., 1997; Sellström et al., 1998; Johnson and Olson, 2001; Ikonomou et al., 2002; de Boer et al., 2003). The most abundant congener in sediment samples is generally the deca-brominated compound (BDE-209) (Watenabe et al., 1987; Sellström et al., 1998; de Boer et al., 2003). Other PBDE homolog groups i.e., the hepta-, octa-, and nona-BDEs, are not as routinely analyzed or reported, so environmental levels of these congeners are not as thoroughly documented. However, studies have shown the presence of hepta- and octa-BDEs in sediments (Sakai et al., 2002) and fish (Rice et al., 2002).

Although tetra- to hexa-brominated congeners tend to dominate the patterns observed in wildlife, the pentabrominated product, which contains mainly tetra- and penta-brominated congeners, is a minor portion of the commercial PBDE flame retardant market. In 2001, production of commercial penta-BDE formulations, which contain predominantly BDE-47 and -99, accounted for 11% of the world-wide PBDE market, and 95% of the penta-BDE was used in the Americas; production of octa- and deca-BDE mixtures accounted for 6% and 83% of the market, respectively (www.bsef.com). A typical commercial octa-BDE mixture contains mainly hepta- and octa-BDEs (70–80%) with little or no tetra-BDE and small amounts of penta- and hexa-BDEs (11%) (World Health Organization, 1994). The commercial deca-BDE product is over 97% pure (World Health Organization, 1994). The predominance of lower brominated congeners in biota (especially BDE-47) may be due to preferential bioavailability and bioaccumulation of these PBDEs, de bromination of the higher brominated congeners, or factors such as exposure, transport and stability in the environment. In order to examine the bioavailability and bioaccumulation of PBDEs at low exposure levels, we have performed a mass balance study in male rats fed a low dose of a commercial penta-BDE mixture for 21 days.

2. Materials and methods

A commercial penta-BDE mixture (DE-71; Great Lakes Chemical, West Lafayette, IN) dissolved in peanut oil served as the dose for the 21-day feeding study. The rat feed (Rat Diet, PMI Nutrition International, LLC, Brentwood, MO) contained not less than 22% crude protein, 4% fat, and 5% fiber was purchased from a local vendor and ground in-house. Male Sprague–Dawley rats (n = 16; 268 ± 18.9 g; 56 days old; Taconic Labs, Germantown, PA) were trained for 16 days to consume the ground rat feed topped with peanut oil within a 1 h time window. During this training period, an optimal amount of feed to provide weight maintenance and appetite satiation was determined to be 11 g rat⁻¹ day⁻¹. The rats were randomly divided into two groups of eight: dosed and control. All animals were fed 200 μl of peanut oil in 11 g of feed for 21 days. The dosed rats received 37 ng DE-71 in the daily oil ration for a total dose of approximately 760 ng of the DE-71 formulation per rat. The rats were housed individually in stainless steel metabolism cages, which allowed for separation of urine and feces, and the room was kept at 25 °C with a 12 h light:12 h dark cycle. The rats were killed 24 h after the last feeding; feces, livers and carcasses were collected and frozen at −70 °C until analyzed.

Each carcass from the dosed rats was homogenized separately in a Hobart grinder. Each liver was diced to homogeneity with a razor blade. All feces were collected during the three-week dosing period, combined for individual rats, and lyophilized. Urine from each of the dosed rats was collected for the duration of the dosing period and pooled. For the control rats, the respective tissues, feces, and urine were pooled before processing. The ground rat feed and peanut oil used for the feeding study were analyzed as used.

All solid samples (~10 g) were spiked with six 13C-labeled PBDE recovery standards (13C-BDEs-28, -47, -99, -153, -154, and -183) (Wellington Laboratories, Guelph, ON) prior to extraction and cleanup. The wet tissue samples were ground with Celite and extracted in an Accelerated Solvent Extractor (ASE) (Dionex Corp., Sunnyvale, CA) with 50:50 hexane:methylene chloride at 100 °C and 1500 psi. The dried feces and ground rat feed were extracted under the same conditions without Celite. The extracts were purified by a modification of EPA Method 1613B (tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS, 10/1994) including sequential washing of the extracts with 20% aqueous potassium hydroxide, water, concentrated sulfuric acid, and water, followed by chromatography on an automated Dioxin Prep system (FMS) (Fluid Management Systems, Inc., Waltham, MA) using triphasic silica and basic alumina columns. After elution from the alumina column, samples were concentrated, applied to a Norit-A/Celite column (0.7 × 2.5 cm), and eluted as described in Method 1613 collecting the reverse toluene elution. The feces samples were further purified on Florisil columns (4.5 g) as follows: the columns were pre-washed with 10 ml hexane, the sample was applied in 1 ml hexane, impurities were eluted with 60 ml hexane, and PBDEs were eluted with 25 ml 10% ether in hexane.

Urine samples (25 ml) were spiked with the 13C-labeled PBDE recovery standards and then partitioned with
saturated ammonium sulfate, ethanol, and hexane. The hexane phase was subsequently extracted with concentrated sulfuric acid and water before purification by column chromatography on the FMS and on a Norit-A/Celite column, as described above. Peanut oil (1 g) was applied directly to the FMS system for purification followed by the Norit-A/Celite column.

GC–MS analyses were performed on a Hewlett Packard 5890 gas chromatograph (Agilent Tech., Wilmington, DE) coupled to a VG Autospec instrument (Micromass, Beverly, MA) operating in the electron impact selective ion monitoring mode at a resolution of 5000 (Huwe et al., 2002a). PBDEs were quantitated using an isotope dilution method by comparison to an internal standard (13C-BDE-77 for mono- to tetra-BDEs, 13C-BDE-139 for penta- to deca-BDEs) and correction for recovery of the nearest eluting 13C-labeled recovery standard. A five-point standard curve was generated from a mixture of 40 native PBDEs in nonane over the following ranges: mono- to tetra-BDEs, 5–75 pg µl⁻¹; penta-BDEs, 7.5–113 pg µl⁻¹; hexa-BDEs, 10–150 pg µl⁻¹; hepta-BDEs, 12.5–188 pg µl⁻¹. For the major dosed congeners, detection limits were determined as the mean plus three standard deviations of the levels found in the method blanks. Typical detection limits for samples ranged from 5 ppt (BDE-28) to 140 ppt (BDE-99).

The PBDE congener composition of the DE-71 formulation was also determined by the isotope dilution GC–MS method (Table 1). The background PBDE levels measured in the control rats were subtracted from the levels in the dosed rats to attempt a mass balance calculation for congeners in the DE-71 dose.

The quality of the analytical method was validated by spiking known amounts of the 40 native PBDEs into control carcass, liver, and feces and analyzing in replicate (N = 3–6). After background subtraction, the major PBDEs (28, 47, 99, 100, 153, 154, and 183) were quantitated in carcass and liver with a precision (RSD) and accuracy (% error) better than 30%. In feces, the precision and accuracy of BDE-47 was somewhat worse (36% and 40%, respectively), and the accuracy of BDE-100 was 50%. The quantitation of BDE-85 could not be validated by this method. The recoveries of 13C-labeled surrogates were generally between 25% and 100%. If recoveries fell below 10%, values were not used.

Hydroxylated metabolites were qualitatively identified by a modification of the method reported by Hovander et al. (2000) for plasma. Briefly, ASE extracts were concentrated and partitioned between hexane and potassium hydroxide (0.5 M in 50% ethanol). The basic fraction was acidified, extracted with ether:hexane (50:50), and the organic phase was derivatized with a diazomethane solution that had been prepared in-house from Diazald according to the manufacturer’s directions (Sigma–Aldrich Co., Milwaukee, WI). After derivatization, lipids were removed by extraction with concentrated sulfuric acid. The metabolites were further purified on a sulfuric acid:silica column (0.7:1, w/w, 2.0 g) by eluting with hexane:methylene chloride (50:50, 30 ml) and then on a neutral alumina column (3.0 g) by rinsing with hexane (20 ml) and finally eluting the metabolites with hexane:methylene chloride (90:10, 30 ml).

The methoxylated BDEs were analyzed on the same instrument as the PBDEs using 13C-labeled BDE-77 and 139 as internal standards and selectively monitoring M + 2 and M + 4 ions for 13C-tetraBDEs, methoxy-tetraBDEs, and methoxy-pentaBDEs. One commercial standard, 4′-hydroxy-2,2′,4,5′-tetraBDE (4′-OH-BDE-49) (Cambridge Isotope Laboratories, Andover, MA), and two synthetic standards, 6-hydroxy-2,2′,4,4′-tetraBDE (6-OH-BDE-47) and 5-hydroxy-2,2′,4,4′-tetraBDE (5-OH-BDE-47) (gifts from Dr. Bergman, Stockholm University, Stockholm, Sweden) were available for comparison to isolated metabolites. Several other metabolites were available as isolates from previous metabolism studies with 14C-BDE-99 (Hakk et al., 2002), 14C-BDE-100 (Hakk et al., 2006), and 14C-BDE-154 (Hakk et al., 2005). The qualitative identification of metabolites from this study was based on the presence of both selected ions, a ratio of those two ions within 15% of theoretical, and a retention time within 1 s of a known metabolite. Because of the lack of standards to accurately assess recoveries and provide quantitation, only semi-quantitative results were obtained for 4′-OH-BDE-47 (not corrected for recovery) based on a five-point standard curve from 10 to 5000 pg 4′-CH₃O-BDE-47/µl. The typical recovery of 4′-OH-BDE-49 spiked into control feces was 84 ± 3.3%.

3. Results

Each dosed rat received DE-71 in the daily feed at a total concentration of approximately 3 ng/g wet feed weight. No visible adverse effects were observed for the group of dosed rats. During the training period of the experiment all rats lost weight. Subsequently, during the dosing phase when feed intake remained restricted to 11 g day⁻¹, the rats gained an average of 5 g each in the control group and 9 g each in the dosed group. We believe this difference in weight gain is attributable to the fact that the control group weighed more on average (249 g) than
the dosed group (241 g) at the beginning of the dosing period and, therefore, required more of the restricted diet to maintain their weight rather than grow. The final average weights of the control and dosed groups were 254 ± 9.8 g and 250 ± 9.9 g, respectively.

The recoveries of the 13C-labeled standards differed with each matrix cleanup procedure but, on average, ranged from 32% to 64% for carcasses, 39–65% for livers, 14–43% for feces, and 28–69% for urine. In a few cases, recovery standards and/or native analytes were obscured by interfering compounds even after rigorous cleanup attempts and were not included in the final data. Due to multiple extraction and chromatography steps, PBDEs that did not have a corresponding 13C-labeled surrogate (such as BDE-85) could not always be reliably quantitated and, therefore, are not included in the results.

PBDEs were detected in all of the samples; however, the control rats had lower levels of the major congeners than the dosed rats (Table 2). In addition to six major PBDE congeners, several other minor congeners were detected in both the control and dosed rats. These congeners included an unidentified tri-BDE, BDE-155, BDE-85, BDE-183, and BDE-209. The background levels measured in the control rats were most likely due to air-born or method contaminations, and trace amounts accumulating from the feed or peanut oil. The feed and peanut oil were analyzed, and low PBDE levels were found that may have contributed an additional 12% to the total dose over the 21 days (Table 1). Because this additional amount was common to both control and dosed rats, a correction for the mass balance calculation was done by subtracting the control rat levels from the dosed rat levels. A pooled urine sample from the dosed group and one from the control group were analyzed, but no significant amounts of PBDEs were found (data not shown).

The majority of the dosed PBDEs were found in the liver, carcass, and feces. Tissue retention and fecal excretion in the dosed rats for the five most abundant BDE congeners in the DE-71 formulation are shown in Table 3 and Fig. 1. After 21 days of dosing, an average of 0.2–0.9% of the dosed congeners remained in the livers and 25–49% remained in the carcasses. Overall, 37–59% of the dosed congeners were accounted for in the livers, carcasses, and excreted feces. Urine may have contained, at most, <0.3% of the dose.

Hydroxylated metabolites were found in the highest concentration in the feces with lesser amounts in the carcass. The livers were not analyzed for metabolites, because no tissue remained after the analysis of the PBDEs. In all, six hydroxylated metabolites were identified as methoxy ether derivatives by GC–MS and included three hydroxy-tetra-BDEs and three hydroxy-penta-BDEs (Fig. 2).

### Table 2

<table>
<thead>
<tr>
<th>PBDE</th>
<th>Method blanks</th>
<th>Control liver</th>
<th>Dosed liver</th>
<th>Control carcass</th>
<th>Dosed carcass</th>
<th>Control feces</th>
<th>Dosed feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 4)</td>
<td>(n = 8)</td>
<td>(n = 4)</td>
<td>(n = 8)</td>
<td>(n = 5)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>28/33</td>
<td>2.3 ± 0.8</td>
<td>5.4 ± 2.4a</td>
<td>5.2 ± 1.6b</td>
<td>6.6 ± 1.3c</td>
<td>6.7 ± 1.7</td>
<td>16 ± 7.7</td>
<td>12 ± 0.8</td>
</tr>
<tr>
<td>47</td>
<td>65 ± 15</td>
<td>184 ± 51a</td>
<td>375 ± 78b</td>
<td>227 ± 21a</td>
<td>621 ± 125</td>
<td>454 ± 147</td>
<td>708 ± 127</td>
</tr>
<tr>
<td>99</td>
<td>95 ± 16</td>
<td>175 ± 105</td>
<td>499 ± 129</td>
<td>114 ± 28</td>
<td>952 ± 295</td>
<td>254 ± 90</td>
<td>1264 ± 342</td>
</tr>
<tr>
<td>100</td>
<td>6.7 ± 2.5</td>
<td>22 ± 4.0</td>
<td>86 ± 17</td>
<td>34 ± 4.6</td>
<td>157 ± 44</td>
<td>55 ± 18</td>
<td>170 ± 69</td>
</tr>
<tr>
<td>153</td>
<td>10.6 ± 6.7</td>
<td>20 ± 5.4</td>
<td>101 ± 21</td>
<td>33 ± 5.9</td>
<td>140 ± 38</td>
<td>29 ± 10</td>
<td>204 ± 43</td>
</tr>
<tr>
<td>154</td>
<td>7.5 ± 1.8</td>
<td>10 ± 7.1</td>
<td>22 ± 6.9</td>
<td>8 ± 1.3</td>
<td>45 ± 14</td>
<td>30 ± 6</td>
<td>133 ± 31c</td>
</tr>
<tr>
<td>Lipid %</td>
<td>1.19 ± 0.28</td>
<td>0.94 ± 0.6</td>
<td>2.5 ± 0.53</td>
<td>2.3 ± 0.44</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

Liver and carcass concentrations are expressed on a wet weight basis; feces concentrations are on a dry weight basis. Control values are averages of four or five replicate analyses of the control rat pools. Dosed values are averages based on the analysis of eight individual rats. nd = Not determined.

a Values based on n = 3 due to interferences.

b Values based on n = 7 due to interferences and low recovery.

c Values based on n = 6 due to interferences and low recovery.

### Table 3

Total amount of dose (ng) and average control-subtracted amounts of five major PBDEs (ng ± sd) in liver, carcass, and feces of male rats administered a commercial DE-71 formulation for 21 days

<table>
<thead>
<tr>
<th>PBDE</th>
<th>Total dose</th>
<th>Amount in liver</th>
<th>% of Dose</th>
<th>Amount in carcass</th>
<th>% of Dose</th>
<th>Amount in feces</th>
<th>% of Dose</th>
<th>Total % recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>230</td>
<td>1.2 ± 0.56a</td>
<td>0.5a</td>
<td>90.8 ± 30.7</td>
<td>39.5</td>
<td>10.3 ± 4.8</td>
<td>4.5</td>
<td>44.5</td>
</tr>
<tr>
<td>99</td>
<td>370</td>
<td>2.8 ± 0.98</td>
<td>0.7</td>
<td>181.9 ± 72.7</td>
<td>49.2</td>
<td>34.0 ± 10.1</td>
<td>9.2</td>
<td>59.1</td>
</tr>
<tr>
<td>100</td>
<td>60</td>
<td>0.5 ± 0.18</td>
<td>0.8</td>
<td>25.3 ± 11.5</td>
<td>42.2</td>
<td>3.8 ± 1.8</td>
<td>6.4</td>
<td>49.4</td>
</tr>
<tr>
<td>153</td>
<td>70</td>
<td>0.6 ± 0.16</td>
<td>0.9</td>
<td>23.6 ± 7.4</td>
<td>33.8</td>
<td>6.0 ± 1.3</td>
<td>8.5</td>
<td>43.1</td>
</tr>
<tr>
<td>154</td>
<td>30</td>
<td>0.06 ± 0.07</td>
<td>0.2</td>
<td>7.6 ± 3.3</td>
<td>25.3</td>
<td>3.5 ± 0.6b</td>
<td>11.7a</td>
<td>37.2</td>
</tr>
</tbody>
</table>

For liver, carcass, and feces n = 8, except where noted.

a Values based on n = 7 due to interferences and low recovery.

b Values based on n = 6 due to interferences and low recovery.
The dose of PBDEs used in this study (3 ng/g in the feed) was designed to mimic a low-level environmental exposure but still provide sufficiently elevated levels of PBDEs in the animal tissues to make detection reliable. Because of the expense, time, and resources involved in the PBDE analysis, control samples were pooled to minimize the number of samples required for the study. For this same reason, only one aliquot of composite urine was analyzed because the lipophilic nature of these compounds and previous dosing studies indicated that PBDEs would not be preferentially found in urine (Örn and Klasson-Wehler, 1998; Hakk et al., 2002). The PBDEs from the DE-71 dose were readily bioavailable. Almost one-half of the dose remained in the carcasses of the rats after the 21 days of feeding. The liver was a minor depot (<1% of the dose) and did not concentrate PBDEs to any greater extent than the remaining carcass on a wet-weight or lipid-weight basis. These results parallel observations made in a limited number of metabolism studies in rats that showed high bioavailability and tissue retention for the tetra- to hexa-brominated congeners. Three days after single oral doses of 14C-BDE-99, -100, or -154 to rats, Hakk et al. (2002, 2005, 2006) found approximately 1% of each dose in the liver and 50%, 70%, and 30%, respectively, in the remaining carcass. Örn and Klasson-Wehler (1998) saw somewhat higher levels of radioactivity (86% of the dose) remaining in the bodies of rats five days after receiving a single dose of 14C-BDE-47; 0.5% of the dose remained in the livers. In all cases, the parent BDEs were the main 14C-compounds extracted from the tissues.

As in the previous studies, the major route of excretion for the PBDEs in the present experiment was in the feces. The feces contained 5–12% of the dosed PBDEs; urine contained <0.3%. Cumulative amounts in the liver, carcass, and feces totaled over 50% of the dose, leaving approximately half of the dose unaccounted for. This imbalance suggests that significant metabolism may have occurred for the major congeners. For BDE-99, 40% of the dose was not recovered and, therefore, may have been metabolized. Somewhat lower amounts of metabolites and inextractable residues were observed by Hakk et al. (2002) accounting for approximately 17% of a single 14C-BDE-99 dose. For BDE-47 in the present study, 57% of the dose may have been metabolized. This is much higher than reported by Örn and Klasson-Wehler (1998) who observed only a limited amount of metabolism of BDE-47 in male rats (3%) after a single oral dose. For BDE-154, the percents of unrecovered parent were similar in this study and a previous one utilizing a single dose (Hakk et al., 2005): 63% and 54%, respectively.

Several differences in study designs may explain the apparently higher rates of metabolism for some of the congeners in this present study. A larger dose in the earlier studies (9–14 mg kg body weight−1 vs. approximately 120 ng kg body weight−1 day−1 in this study) may have overwhelmed metabolizing systems leading to more excretion or storage of the parent compounds. Chronic dosing (21 d), in contrast to a single dose, may have induced metabolizing enzyme systems. The induction of P-450 enzymes has been demonstrated with penta-BDE formulations, and repeated dosing has been found to be more effective at induction than a single dose (Von-Meyerinck et al., 1990; Fowles et al., 1994). However, enzyme induction was not measured in our study, and the low level of penta-BDE formulation used was below the dosages previously investigated and shown to induce enzyme activity. BDEs-47, -99, -100, and -154 are metabolized to hydroxylated compounds in rats, products indicative of P-450 oxidation (Örn and Klasson-Wehler, 1998; Hakk et al., 2002, 2005, 2006). Metabolism can also lead to the formation of non-extractable bound residues. The proportion of bound residues was shown to increase in the feces with time.
following single doses of several PBDs (Örn and Klasson-Wehler, 1998; Hakk et al., 2002, 2006). Continuous dosing and exposure may have increased the amounts of metabolites or bound residues formed in the present study. Although recoveries of some of the PBDs were low, we believe this was due to the extensive (and not fully optimized) purification methods used by our laboratory at the time, not incomplete extraction, because these same extraction methods have been demonstrated to be reliable for the extraction of other lipophilic compounds, such as polychlorinated dioxin and furans, from liver and tissue (Huwe, 2002b; unpublished data). In addition, quantitation of each native PBD was corrected for recovery using 13C-labeled surrogates; therefore, metabolism to bound or free conjugates seems to be a better explanation for the unrecovered portion of the DE-71 dose.

Although metabolism resulting in bound residues can not easily be measured in this study, at least six hydroxylated metabolites were qualitatively identified in the feces of the rats (Fig. 2). These metabolites corresponded to metabolites previously identified in studies with 14C-BDEs-99, -100, and -154 as mono-hydroxylated BDEs (Hakk et al., 2002, 2005, 2006). Two of the metabolites were tentatively identified as 6-OH-BDE-47 and 4’-OH-BDE-49 by retention time comparison of their methyl ethers to standards (17:38 and 17:91 min, respectively). Both of these hydroxylated metabolites have previously been identified in the feces of rats fed BDE-47 (Marsh et al., 2006) and also correspond to metabolites found in the feces of rats dosed with either BDE-100 (6-OH-BDE-47) or BDE-99 (4’-OH-BDE-49). Another possible hydroxy-tetraBDE was observed by GC/MS (18:63 min), but no standard was available to confirm its identity. Three hydroxy-pentaBDEs were found that correlated to the major fecal metabolites isolated from rats dosed with BDE-99 (metabolites at 19:53 and 20:25 min) or with BDE-100 (metabolite at 19:02 min). A possible hydroxy-hexaBDE corresponding to a BDE-154 fecal metabolite was seen at 20:40 min (Fig. 2). Feces from the control rats were analyzed and contained some of the same hydroxyl metabolites but at approximately 3–10 times lower levels.

A semi-quantitative estimate of the total amount of 4’-OH-BDE-49 excreted in the feces of each dosed rat was 5.0 ± 1.4 ng or <1% of the DE-71 dose. Even if we assume similar amounts of the other five metabolites, this amount does not account for the remaining mass balance which was approximately 48% of the dose. The carcasses of the dosed rats contained some of the same hydroxylated metabolites as seen in the feces but at 10–80 times lower concentrations and would account for only a fraction of the DE-71 dose (estimated < 5%). Liver was not analyzed for metabolites, but has previously been shown to contain only trace amounts of extractable PBD metabolites (Örn and Klasson-Wehler, 1998; Hakk et al., 2002, 2006). Although methoxy-BDEs have been identified in marine systems (Haglund et al., 1997; Vetter, 2001; Varreault et al., 2005), methoxy-BDE metabolites have not been

found in rats dosed with BDEs-47, -99, -100, or -154 (Örn and Klasson-Wehler, 1998; Hakk et al., 2002, 2005, 2006) and, therefore, were not investigated as a major sink for parent PBDs. The lack of a balanced mass recovery suggests extensive metabolism of these PBDs and leaves further work to be done on quantitating and identifying all metabolites, measuring enzyme activities, and designing experiments to investigate bound residues that may form during chronic PBD dosing.

For the PBDs analyzed in this study, neither bioavailability nor bioaccumulation were significantly different between the groups of homologs (Figs. 1 and 3). If the amount of parent compound excreted in the feces is regarded as the unavailable portion of the dose, then the bioavailabilities ranged from 95% for the tetra-BDE (47) to 88% for a hexa-BDE (154). Uptake in rats was not particularly selective between or within the tetra-, penta-, or hexa-BDE homolog groups. In the same manner, accumulation in the carcass and liver was not homolog-dependent. The congeners patterns in the liver and carcass remained remarkably similar to that of the original dose (Fig. 3). No evidence of debromination to lower brominated compounds was suggested from the data.

These results differ from the results of studies in aquatic species. Bureau et al. (1997) observed uptake in pike decreasing with the degree of bromination from 90% for BDE-47 to 60% for BDE-99 to 40% for BDE-153. BDE-47 has also been shown to accumulate to a higher degree than penta- or hexa-BDEs in carp (Stapleton et al., 2002) and zebrafish (Andersson et al., 1999). Baltic Sea blue mussels accumulated BDE-47 and -99 at similar rates, but BDE-153 was accumulated at only one-tenth the rate (Gustafsson et al., 1999). In each of these studies, whole organisms were analyzed for PBDs after an exposure, but no attempts were made to measure excretion or metabolism in order to provide a mass balance. Differences in the accumulation of various congeners could be the result of different metabolic activities not merely different uptake efficiencies. For example, the total absence of BDE-99 in carp tissue after an exposure has been attributed to
metabolic biotransformations including debromination to BDE-47 (Stapleton et al., 2004).

In conclusion, the results from this 21-day dosing experiment in rats do not show any obvious differences between the bioavailability or bioaccumulation of lower brominated congeners, especially the most common tetra- through hexa-PBDEs. The congener distribution pattern in the rat carcasses and livers was the same as in the DE-71 dose. A congener pattern resembling a penta-BDE formulation has also been reported in chickens and in domestic meat samples; however, the source of the PBDEs in these food samples is unknown (Huwe et al., 2002a; Schecter et al., 2004; Huwe and Larsen, 2005). Data from the present study are quite different than data collected in studies on fish where BDE-47 was more readily accumulated in fish tissue than other PBDEs (Burreau et al., 1997; Andersson et al., 1999; Stapleton et al., 2002). Selective uptake and metabolism in fish and other aquatic organisms may also explain the observed higher proportion of BDE-47 in wildlife, such as birds of prey, seals, and whales, relative to other PBDEs in commercial products (de Wit, 2002; Law et al., 2003). That is, fish-eating mammals and birds may display a congener pattern similar to the food they eat (i.e., fish), just as the rats in this study did. In addition to inherent differences between species, factors such as the dose amount, the length of exposure, the exposure matrix, and the environmental transport and stability of different congeners may influence PBDE body burdens in wildlife and laboratory and domestic animals.

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References


