DECABROMODIPHENYL ETHER IN THE RAT: ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

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ABSTRACT:

Among the group of polybrominated diphenyl ethers used as flame-retardants, the fully brominated diphenyl ether, decabromodiphenyl ether (decaBDE), is the most commonly used. Despite the large usage of decaBDE, neither the metabolic pathways nor the absorption have been addressed, and there are very few studies on its toxicology. In this work, it is shown that after a single oral dose of $^{14}$C-labeled decaBDE to rats, at least 10% of the decaBDE dose is absorbed. The major excretion route in conventional rats is via feces that contained 90% of the decaBDE dose. The excretion in bile was close to 10% of the dose and represented mainly metabolites. It cannot be excluded that greater than 10% of the oral dose had been absorbed since 65% of the radioactivity excreted in feces was metabolites. The highest concentrations on a lipid weight basis were found in plasma and blood-rich tissues, and the adipose tissue had the lowest concentration of decaBDE. After derivatization of a phenolic fraction, gas chromatography-mass spectrometry (GC/MS) analyses indicated that metabolites with five to seven bromine atoms had formed, and they possessed a guaiacol structure (a hydroxy and a methoxy group) in one of the rings. In addition, traces of nonabrominated diphenyl ethers and monohydroxylated metabolites were found by GC/MS. Metabolites, characterized by their chemical properties, were interpreted to be covalently bound to macromolecules, either proteins or lipids. In addition, water solubility was suggested. The metabolic pathway was indicated to include a reactive intermediate.

Polybrominated diphenyl ethers (PBDEs1) belong to the large family of brominated flame-retardants, which are used as additives in polymers and textiles. The fully brominated ether decabromodiphenyl (decaBDE) is currently the most common PBDE, with a worldwide production of approximately 54,800 tons in 1999 [Bromine Science Environmental Forum, Brussels, Belgium (2000), http://www.bsef.com]. DecaBDE and lower brominated PBDEs have been found in biotic and abiotic environmental samples ranging from river sediments to wildlife such as the peregrine falcon (de Boer et al., 2000; Sellström et al., 2001; de Wit, 2002). In addition, humans are also exposed, as evidenced by the detection of decaBDE in blood from occupationally exposed and unexposed individuals (Sjödin et al., 1999, 2001).

DecaBDE has an extremely low solubility in water and is also limited in organic solvents [e.g., 2 mg/ml toluene; WHO (1994)]. The gastrointestinal tract readily absorbs lipophilic compounds with molecular weights up to 350 by passive diffusion (Rowland and Tozer, 1980). DecaBDE has a molecular weight of 959; therefore, it should not be easily absorbed in the gut. Active transport via proteins can explain absorption of compounds with low bioavailability and high molecular weight (Tsuij and Tamai, 1996; Charman, 2000). Studies of decaBDE in rat indicated that oral absorption from the diet was very low (Norris et al., 1975; El Dareer et al., 1987).

Studies of $^{14}$C-labeled decaBDE in the rat showed a high elimination rate following repeated oral and single i.v. administrations (El Dareer et al., 1987). Ninety-nine percent of the oral and 70% of the i.v. dose were recovered in feces within 72 h. The excreted radioactivity consisted mainly of metabolites, but none were identified.

Recent studies of decaBDE have shown that it is irreversibly neurotoxic, causing cognitive deficiencies in mice exposed in utero (Viberg et al., 2001). There are a limited number of studies on the toxicity of decaBDE, but the lower polybrominated diphenyl ether congeners have been studied more extensively (Darnerud et al., 2001). Permanent aberrations in the spontaneous behavior in adult mice after neonatal exposure have been reported for 2,2’,4,4’-tetrabromodiphenyl ether (tetrabDE) and 2,2’,4,4’,5-pentabromodiphenyl ether (pentaBDE) (Eriksson et al., 2001). Interactions with the thyroid hormone system (Meerts et al., 2000), induction of CYP1A1 and CYP1A2 (von Meyerinck et al., 1990; Hanberg et al., 1991; Chen et al., 2001), and agonistic and antagonistic activity toward the aryl hydrocarbon receptor (Meerts et al., 1998) are examples of biological effects caused by penta-, hexa-, and octabrominated diphenyl ethers. DecaBDE has been classified as showing “some evidence of carcinogenicity” by the...
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National Toxicology Program of the U.S. Department of Health and Human Services (National Toxicology Program, 1986).

The aim of the present study was to investigate the metabolism of decaBDE, focusing on intestinal absorption and the metabolic pathways. Comparisons will be made to previously studied lower brominated diphenyl ethers (Örn and Klasson Wehler, 1998; Hakka et al., 2002). Previous studies on decaBDE metabolism have shown low absorption at various levels in diet (8 mg/kg b.wt/day for 30 days; Norris et al., 1975; and 0.025–5% of the diet for 14 days; El Dareey et al., 1987). In contrast, tetraBDE and pentaBDE were both readily absorbed from the gut after a single oral dose (15 mg tetraBDE/kg b.wt. and 9 mg pentaBDE/kg b.wt.; Örn and Klasson Wehler, 1998; Hakka et al., 2002). The tetraBDE was metabolically stable, with less than 14% of the dose excreted in feces, primarily as parent compound. Less than 50% of the pentaBDE dose was recovered in feces (<1% in urine), mostly as parent compound.

To improve absorption when compared with previous studies of decaBDE, emphasis was placed on formulation of the dose in a novel vehicle to enhance decaBDE solubility, without exceeding the level used for tetraBDE and pentaBDE studies. Bile duct-cannulated rats were included in the present study to observe elimination via the bile and to compare metabolic profiles in the bile and feces. Tissue distribution was determined by various radioactivity measurements.

Metabolites were analyzed in excreta and tissues by their behavior on gel permeation chromatography (GPC) columns and by gas chromatography-mass spectrometry (GC/MS).

Materials and Methods

Chemicals. The chemicals used and their purity grade are as follows: n-hexane and dichloromethane (HPLC grade; Fisher Scientific, Leicestershire, UK), methanol and chloroform of p.a. grade from Merck (Darmstadt, Germany) and methyl tert-butyl ether (HPLC grade; Ratburn Chemicals, Walsburn, Scotland, UK), acetone (p.a. grade from Riedel de Haen, Seelze, Germany), acetic anhydride and 2-propanol (p.a. grade) from ProLabo (Cedex, France), triethyl amine (p.a. grade) from Fluka (Buchs, Switzerland). Potassium hydroxide (p.a. grade; Eka Nobel, Bolus, Sweden). Silica gel 60 (0.063–0.200 mm), sulfuric acid, and hydrochloric acid (p.a. grade) and citrate-hydrochloric acid buffer (pH 4) obtained from Merck were used. Diazomethane was synthesized according to the method described by Fieser and Fieser (1967).

Synthesis of decaBDE. [14C]Phenol (specific activity 35.0 Ci/mol) was brominated with bromine (2 Eq) to 2,4-dibromophenol, which was reacted with 2,2',4,4'-tetrabromodiphenyldiosionium salt to tetraBDE as described by Marsh et al. (1999). The specific activity of the product was diluted with unlabeled tetraBDE to 17.5 Ci/mol. The [14C]tetraBDE was fully brominated with bromine (approximately 25 Eq) using AlBr3 as a catalyst. decaBDE was isolated by silica gel thin-layer chromatography of high purity (>98%, specific activity 17.5 Ci/mol) with dichloromethane as mobile phase. The radioactivity was detected using a Raytest RITA 3200 TLC scanner (gas: 10% methane in nitrogen). The activity 17.5 Ci/mol of decaBDE was measured after 3 days and the remaining four rats after 7 days (conventional rats). Two additional rats (200 g) were subjected to bile duct cannulation (bile duct-cannulated rats), as described elsewhere (Larsen and Bakke, 1981), but otherwise were treated the same as the conventional rats prior to sacrificing them at 3 days.

All rats were dosed orally by gavage with 14C-labeled decaBDE (3 μmol/ kg, 15 Ci/mol, dose volume 1 ml/kg). Urine and feces were collected at 24-h intervals for 3 and 7 days, respectively. From the conventional rats, liver, adiopose tissue, lung, kidney, adrenals, skin, muscle, spleen, testis, thymus, heart, plasma, colon wall and contents, small intestine contents, and small intestine wall were collected. Tissues from bile duct-cannulated rats were collected but not further processed. Bile from the bile duct-cannulated rats was collected after 4, 12, 24, 48, and 72 h postdosing and stored at −18°C. All samples were stored at −18°C until further analysis.

The feces, bile, liver, and adipose tissue, were extracted individually. The remaining tissues were pooled samples generated for each group prior to extraction. The extracts for liver, lung, kidney, small intestine wall, adipose tissue, bile and feces were further processed and the radioactivity was partitioned into various metabolic fractions according to the method described in Fig. 1. The metabolites from feces, bile, liver, kidney, lung, adipose tissue and small intestine wall in the three-day conventional rat group, were further analyzed by GC/MS.

Instruments. All radioactivity measurements were performed on a Wallac 1409 liquid scintillation counter using emulsifier scintillator 299 (PerkinElmer Life Sciences, Boston, MA) and Opti Scint Hisaf 2 (PerkinElmer Wallac, Turku, Finland) as scintillation cocktails. Aqueous samples (1 ml) were directly mixed with scintillation cocktail. Freeze-dried fecal samples (approximately 20 mg dry weight) were moistened with 100 μl of water for 30 min before digestion in Soluene-350 (PerkinElmer Life Sciences, 1 ml) for 4 h, until fully dissolved. Tissue samples (approximately 100 mg wet weight) were digested in Soluene. Hydrogen peroxide/2-propanol (2:3, v/v, 1 ml) was added.
and the samples were stored in the dark for 2 h before measurement. The scintillation counter function for automatic compensation for chemiluminescence was applied.

The GC/MS analyses were performed on a Finnigan TSO 700 instrument (Thermo Finnigan, Bremen, Germany), connected to a Varian 3400 gas chromatograph equipped with a DB-5HT capillary column (15 m, 0.25-mm i.d., 0.1-μm film thickness; J&W Scientific Inc., Folsom, CA). Helium was used as carrier gas (pressure 5 psi). The injections were performed on-column with the injector temperature programmed from 60°C, rate of 150°C/min up to 300°C, and kept there for 10 min. The transfer line temperature was 290°C. The oven temperature was programmed as follows: 80°C (1 min), 15°C/min to 300°C (10 min). The MS instrument was operated in ECNI mode with a primary electron energy of 70 eV using methane (99.995% purity, containing 5 ppm O₂; AGA, Stockholm, Sweden) as the electron thermalization buffer gas. The ion source temperature was 200°C and the pressure was 5.5 torr. Full scans from m/z 30 to 1000 were obtained. For the mass spectral analyses using EI mode, primary electron energy of 70 eV was used, but otherwise, all other parameters were the same.

**Extraction and Cleanup.** The extraction and cleanup of fecal, biliary, and tissue samples used in the present study have been described previously (Klasson Wehler et al., 1996; Möreck et al., 2002). A brief description of the method is given in Fig. 1.

**Feces.** Freeze-dried and homogenized fecal samples were extracted with chloroform/methanol (2:1, 200 ml) in a Soxhlet apparatus (Klasson Wehler et al., 1989). The extract was redissolved in hexane, washed with phosphoric acid (H₃PO₄) (0.1 M in 0.9% NaCl) (Orn and Klasson Wehler, 1998), and the lipid weight was subsequently determined. The radioactivity content in the organic solvent extracts, water phases, and solid residues was determined. The extracts were fractionated by GPC and partitioned with KOH, as described for the fecal samples. Neutral and methylated phenolic metabolites were analyzed by GC/MS (ECNI).

**Bile.** After determination of 14C content, the bile samples were diluted with 1 volume of citrate-hydrochloric acid buffer (pH 4), and the pH was adjusted to 3 with hydrochloric acid (4 M). Lipophilic material was extracted with dichloromethane (three times), as described by Möreck et al. (2002). The lipid weight and radioactivity content were determined. The extracts were fractionated by GPC and partitioned with KOH, as described for the fecal samples. Neutral and methylated phenolic metabolites were analyzed by GC/MS (ECNI).

**Tissues.** Homogenization of the tissues was performed using an Ultra-Turrax IKA homogenizer. The tissues were homogenized in n-hexane/acetone (1:3.5, 45 ml) followed by extraction with n-hexane/methyl-t-butyl ether (9:1, 25 ml), and the combined extracts were washed with phosphoric acid (H₃PO₄) (0.1 M in 0.9% NaCl) (Bergman et al., 1992). The solvent was evaporated and the lipid weight determined gravimetrically. The radioactivity content in extracts, water phases, and residues was determined. The extracts from liver, adipose tissue, kidney, lung, and small intestinal wall were separated by GPC and partitioned with KOH, as described for the fecal samples. The radioactivity content in the neutral and methylated phenolic phases was only determined for the liver samples. The neutral and phenolic metabolites were analyzed by GC/MS using ECNI mode. The neutral metabolites and parent compound were analyzed by GC/MS (ECNI) in full scan mode. Phenolic metabolites were scanned for the bromine isotope masses, m/z 79 and 81.

**Results**

**Solubility of decaBDE.** The solubility (per milliliter) of decaBDE was 3.5 mg in DMSO, 4.1 mg in toluene, 8.8 mg in tetrahydrofuran, 1 mg in peanut oil, 9.3 mg in anisole, <0.8 mg in ethyl acetate, and 6.6 mg in DMA. The highest concentration of decaBDE per milliliter formulation obtained was 7.0 mg in soya phospholipone/Lutrol (16:34, w/w)/water, followed by 3.8 mg in anisole/peanut oil (30:70) and 2.5 mg in DMSO/peanut oil (1:1).

**Excretion.** The dominant route of excretion after the oral dose of decaBDE was via feces, with 90% (86–93%; n = 8) of the dose excreted after 3 days, and only marginally more, i.e., 91% (87–95%; n = 4), after 7 days (Fig. 2). For the two bile duct-cannulated rats, an average of 88% of the dose (62 and 114%) was recovered in feces and 9.5% (11.5–7.5%) of the dose in bile within three days (Fig. 2). The large variation in fecal excretion between these two rats may have been due to the fact that no bile salts were added to compensate for the collected bile. This is likely to have affected the absorption. Urinary excretion of 14C was insignificant for all groups, i.e., <0.1% of the dose.

**Tissue Distribution.** The amount of the 14C dose remaining in the body at 3 and 7 days was approximately 9%, calculated by totaling the urine and feces output and subtracting from 100% (Fig. 2). The highest concentrations of radioactivity (on fresh weight basis) were found in adrenals, kidney, heart, and liver after both 3 and 7 days (Table 1). Based on lipid weight, plasma and liver had the highest
concentrations (Table 1), whereas adipose tissue had low concentrations at both time points. In other tissues, the concentration of radioactivity was low (Table 1).

**Fractionation and Analysis of Metabolites in Feces, Bile, and Tissues.** The radiolabeled material in the samples was characterized by its chromatographic behavior (Fig. 1). The metabolites were thus characterized as nonextractable, water-soluble, lipid bound, phenolic metabolites, and parent compound/neutral metabolites. The total recovery of radioactivity was >95% and the relative distribution in different types of metabolites is presented in Fig. 3a-c.

**Feces.** The relative amount of parent compound and its metabolites at different time points after dosing in conventional rat feces is shown in Fig. 3a. The relative amounts of parent compound decreases by days 2 and 3, whereas the relative amount of lipid bound radioactivity increases by day 3. Compared to the conventional rats, the feces from bile duct-cannulated rat had a much lower fraction of phenolic metabolites and a relatively larger fraction of neutral metabolites per parent compound (Fig. 3b). Fecal phenolic and neutral metabolites in conventional rats were characterized by GC/MS, whereas other metabolites were not further analyzed. The conventional rat fecal phenolic metabolites and the parent compound did not show any molecular ion in GC/MS (ECNI) but were cleaved in two halves at the ether bond (Table 2).

In the neutral fraction, the major component found was decaBDE for all samples, but trace amounts (<0.5% of the decaBDE) of three nonabrominated diphenyl ethers (M1–3) were also indicated. No nonaBDEs were present in the administered decaBDE solution, either at the time of dosing or after storage in the freezer (−18°C) for 12 months (data not shown).

After acetylation of the phenolic fraction, six different metabolites (M5, M6, M9–12), having five to seven bromine atoms, were indicated by GC/MS (ECNI). One of the original phenyl rings was substituted with both a methoxy and an acetylated hydroxy group (Table 2 and Fig. 4). Three monohydroxylated diphenyl ethers with at least six bromine atoms were also indicated as their acetylated derivatives (M4, M7, and M8) (Table 2 and Fig. 4).

Methylation of the phenolic fractions and analysis by GC/MS (ECNI) showed the presence of three additional, but uncharacterized, metabolites having eight bromine atoms. The fragment ions had two or three bromine atoms and an additional substituent with a mass of 58 mass units. This is 2 mass units less than would be expected if the compound was substituted with two methoxy groups. The methylated samples were also analyzed by GC/MS (EI), and seven metabolites containing five to seven bromine atoms were detected (data not shown). As with the fragment in GC/MS (ECNI), the molecular ions were 2 mass units lower than if the metabolites had been dimethoxylated. The mass spectra were thus not consistent with any obvious metabolite.

**Bile.** Lipid-bound metabolites were dominant in bile on day 1, both at 0 to 12 h and 12 to 24 h, but decreased considerably during day 2 and 3 in favor of water-soluble metabolites (Fig. 3c). The neutral and the phenolic metabolites were characterized by GC/MS (ECNI), whereas other metabolites were not further analyzed.

The neutral compounds consist mainly of parent decaBDE and traces of the three nonaBDEs, which were found in feces (M1–M3). An aliquot of the phenolic metabolites was methylated and analyzed by GC/MS (ECNI). Eight metabolites were observed and, according to their full-scan mass spectra and retention times, were the same as those found in feces.

Another aliquot of the biliary phenolic metabolites was acetylated prior to GC/MS analysis, but no acetylated phenolic metabolites could be identified due to lack of purity. Unlike the methylated phenolic metabolites, which were isolated on a silica gel column after methylation, the acetylated phenolic metabolites were not further purified. Pilot experiments showed considerable losses of acetylated metabolites on the silica gel column cleanup (data not shown).

**Tissues.** The radiolabeled material in tissues (liver, lung, kidney, adipose tissue and small intestinal wall) was partitioned, as described for the fecal samples (Table 3). For the liver and small intestine wall, 27% (0.15 nmol/g fw) and 61% (0.04 nmol/g fw) of the radioactivity, respectively, remained in the tissue residue after extraction at day 3 (Table 3). In the liver, 42% of the radioactivity was found in the lipid fraction from GPC and, thus, represented lipid-bound metabolites (Table 3). The nonconjugated metabolites in the liver (30%) were partitioned into phenolic (4%) and neutral metabolites (26%). The neutral compounds were analyzed by GC/MS (ECNI) in full scan mode. The major compound was parent decaBDE and traces (<0.5% of decaBDE) of nonabrominated diphenyl ethers (M1–3) were detected.

Acetylated phenolic metabolites were detected by GC/MS (ECNI), by selected ion monitoring of the bromine isotope masses, m/z 79 and...
Fig. 3. Metabolites in feces.

Shown is the relative distribution of fecal radioactivity in different metabolite groups: neutral and parent decaBDE, phenolic, nonextracted, water-soluble, and lipid-bound metabolites. A, conventional rat (n = 4) feces, 0–72 h; B, bile duct-cannulated rat (n = 2) feces, 0–48 h; C, bile duct-cannulated rat (n = 2) bile, 0–72 h.
Then the full scan chromatograms were compared with the chromatograms of the fecal acetylated phenolic metabolites, and the metabolites were characterized by their relative retention time. The peak pattern for all the tissue samples showed 20 peaks, giving the bromine isotope masses \((m/z 79\) and 81). A retention time comparison suggested that the acetylated phenolic metabolites in the liver and kidney corresponded to the fecal acetylated phenolic metabolites M5 to M11 (Table 2 and Fig. 4). Conversely, the acetylated phenolic metabolites in lung and small intestinal wall corresponded to the fecal metabolites M7 to M10 (Table 2 and Fig. 4), whereas the adipose tissue metabolites corresponded to M7 and M8 (Table 2 and Fig. 4).

**Discussion**

The present paper was mainly focused on qualitative analysis of metabolites formed in rats after a single oral dose with a dose formulation aimed to optimize the absorption. Qualitative data and relative distribution of radioactivity does, however, give valuable data on the metabolism of decaBDE. The low number of animals limits the possibility to evaluate the excretion quantitatively, particularly for the bile duct animals.

The excretion of decaBDE in bile was close to 10% of the dose at 72 h, indicating that at least this much of the oral dose had been absorbed (Fig. 2). It cannot be excluded that a larger part of the dose had been absorbed since 65% of the dose excreted in feces represented metabolized decaBDE (Fig. 3). In contrast, previous studies of decabDE in the rat reported a very low absorption, <1% of the dose (Norris et al., 1975; El Dareer et al., 1987). The decaBDE has a low solubility in most solvents that is appropriate for dose formulations, i.e., nontoxic and relatively nonaqueous. In this study, decaBDE was therefore formulated as an emulsion. Lipophilic compounds with a low molecular weight are easily absorbed by passive transport. For

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**TABLE 2**  
The major GC/MS (ECNI) fragments of acetylated derivatives of phenolic nonconjugated metabolites in conventional rat feces (M4–M12).  
\(x\) and \(y\) represent the sum of bromine atoms.

<table>
<thead>
<tr>
<th>Metabolites listed in order of increasing retention time</th>
<th>Fragment 1</th>
<th>Fragment 2</th>
<th>Fragment 3</th>
<th>Fragment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4</td>
<td>306.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>320.9 (x=2)</td>
<td>326.9 (y=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>320.9 (x=2)</td>
<td>326.9 (y=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M9</td>
<td>320.9 (x=2)</td>
<td>405 (y=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M10</td>
<td>320.9 (x=2)</td>
<td>405 (y=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M11</td>
<td>320.9 (x=2)</td>
<td>405 (y=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M12</td>
<td>399 (x=3)</td>
<td>405 (y=4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not detected.*

**TABLE 3**  
The relative distribution of radioactivity, expressed as percentage of the total radioactivity, in different types of metabolites for liver, lung, adipose tissue, kidney, and small intestinal wall.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Nonconjugated (^{14}\text{C})</th>
<th>Nonextracted (^{14}\text{C})</th>
<th>Water-Soluble (^{14}\text{C})</th>
<th>Lipid-Bound (^{14}\text{C})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>30</td>
<td>27</td>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td>Lung</td>
<td>71</td>
<td>8</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>80</td>
<td>1.5</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Kidney</td>
<td>77</td>
<td>5</td>
<td>0.2</td>
<td>18</td>
</tr>
<tr>
<td>Small intestinal wall</td>
<td>20</td>
<td>61</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Tentative structures of acetylated phenolic metabolites in feces from conventional rat. GC/MS (ECNI) fragmentation of the metabolites M5–12 is presented in Table 2. Metabolite M4 in Table 2 only showed the fragment with the acetylated substituent and two bromine atoms similar to those for M7 and M8.
decaBDE and other lipophilic compounds with a high molecular weight, absorption can be facilitated by transport proteins, such as P-glycoprotein (Rowland and Tozer, 1980; Tsuji and Tamai, 1996; Charman, 2000). The improved absorption in the present study is probably due to the use of a suitable formulation, i.e., soya phospholipone/Lutrol/water.

The excretion of decaBDE in the feces of conventional rats was 90% of the dose, mainly as decaBDE metabolites (i.e., 65% of dose). Ten percent of the dose was excreted via the bile, almost all of which represented metabolites. Assuming that 10% of the dose excreted in feces came from the bile, the remaining metabolites in feces (approximately 55% of the dose; Fig. 3, a and c) had been metabolized elsewhere. The variable excretion in feces in the bile duct-cannulated rats most probably was due to a lower absorption due to the lack of bile acids. The relatively larger fraction parent decaBDE in these samples supports this interpretation (Fig. 3b). After an intravenous administration of decaBDE, the major part of the dose was excreted as metabolites in feces (74% of the dose), but only a small fraction (7%) was excreted via the bile (El Dareer et al., 1987). The presence of fecal metabolites arising from nonbilary sources in both conventional and bile duct-cannulated rats, together with the findings of El Dareer et al. (1987), suggests involvement of active transport proteins. For drugs, active transport proteins have been shown to play an important role in the absorption and/or excretion, affecting the bioavailability (Charman, 2000). For the antiparasitic drug, ivermectin, secretion through the intestinal wall represents the major route of excretion and is mediated by P-glycoprotein (Laffont et al., 2002).

An alternative explanation could be first-pass metabolism by cytochrome P450 enzymes in the intestine wall, similar to that shown for the antiparasitic agent ivermone, where a combination of in vivo, in situ, and in vitro studies was used (Mihara et al., 2001). DecaBDE metabolism occurs in the intestine, which was indicated by the presence of covalently bound radioactivity in the small intestine wall (Table 3). More than 61% of the radioactivity in this tissue was covalently bound, which is a larger fraction than that found in the metabolically active liver (29% of the radioactivity). Still, based on the concentration of radioactivity bound to the tissue, the liver (0.15 nmol/g compared with 0.04 nmol/g intestine) is indicated to be the quantitatively more important tissue for metabolism. Formed metabolites can either be absorbed into the circulation or excreted into the gut lumen, and then be observed as metabolites in feces. However, it cannot be excluded that the gut flora plays a role for some of the metabolites found in feces. The intestinal microflora is known to perform a wide variety of metabolic reactions including bioactivation of several carcinogens (Larsen, 1988; Goldman, 1989; Chadwick et al., 1992). DecaBDE is not readily distributed to adipose tissue (Table 1), and this observation is quite unusual for lipophilic persistent compounds. Many halogenated aromatic compounds readily distribute to lipid-rich tissues. Instead, the highest concentrations of decaBDE were found in plasma and blood-rich tissues, i.e., liver, kidney, heart, and intestinal wall (Table 1). A high plasma protein binding could be an explanation for the slow distribution to adipose tissue. However, plasma protein binding has not been determined for decaBDE. For another perhalogenated aromatic compound, i.e., hexachlorobenzene, low distribution to adipose tissue was explained by binding primarily to the blood lipoprotein, albumin (Gómez-Catalán et al., 1991).

The high concentration of radioactivity in the liver and small intestine wall was in part due to the high amount of nonextractable radioactivity (Table 3). The extraction method used has previously been shown to give high recovery (Klasson Wehler et al., 1989, 1996), and the radioactivity remaining in the tissues was therefore assumed to be bound covalently to macromolecules. Formation of such adducts is indicative of metabolism occurring via reactive metabolites. In the liver, a large fraction of the radioactivity coeluted with lipids on the GPC. Studies of another halogenated aromatic compound, a tetrachloro-phenol, showed that a large fraction of the dose represented metabolites that were covalently bound to lipids, predominantly phospholipids, and were found both in tissues and in excreta (bile and feces) (Mörck et al., 2002).

Characterization of formed metabolites can give information on the metabolic pathway and, indirectly, on the identity of the reactive intermediates. In the nonconjugated phenolic fraction, methoxyhydroxylated penta- to heptabrominated diphenyl ethers were indicated by GC/MS (Table 2 and Fig. 4). The methoxy and hydroxy substituents are on the same phenyl ring, and are proposed to be on vicinal carbons. Methylation, by catechol-O-methyltransferase, of an ortho-catechol, resulting in a guaiacol, would explain the observed metabolites (Kawai et al., 2000) (Fig. 4). The formation of a catechol from a fully brominated aromatic compound is not obvious, since formation of an arenec oxide seems difficult. Debromination may have been the first step, since trace amounts of debrominated metabolites (nona-BDEs) were observed as well as debromination in the other phenoxy ring of guaiacol metabolites. A secondary oxidation of monohydroxylated metabolites is another possibility, although only a small proportion of monohydroxylated metabolites were found in tissues and feces. The initial step in the metabolism of decaBDE is not clear and needs to be further investigated.

Thus, there are possible reactive metabolites, even though only the catechol is obvious. Catechols can be oxidized to quinines as shown for PCBs (Amaro et al., 1996; Lin et al., 2000). Quinone intermediates have been reported to form covalent bonds to macromolecules as a Michael addition product or a Schiff base (Amaro et al., 1996; Bolton et al., 2000; Lin et al., 2000). The same mechanism of Schiff base formation has been reported to result in lipid-bound metabolites (Hazen et al., 1999). The perbrominated hexabromobenzene is also extensively metabolized, but primarily to sulfur-containing metabolites. Debrominated and phenolic, but not guaiacol, metabolites have been reported (Koss et al., 1982; Yamaguchi et al., 1988).

The phenolic metabolites of decaBDE were acetylated and identified by GC/MS using ECNI mode. The metabolites were also analyzed using EI mode, but the regiochemistry of the metabolites could not be determined. The methylated phenolic metabolites gave a better signal-to-noise ratio than did the acetylated metabolites, but the mass fragments observed were 2 mass units less than a dimethoxylated metabolite and were therefore inconsistent with any obvious metabolites.

The metabolism and distribution of decaBDE in the rat are different from those of the lower PBDEs. DecaBDE is extensively metabolized, rapidly excreted, and marginally distributed to adipose tissue. In contrast, tetraBDE is readily absorbed by the rat and distributed to adipose tissue. After 5 days, 86% of the dose remains in the rat, primarily as parent compound in adipose tissue. Approximately 43% of the pentaBDE dose is excreted in feces mainly as parent, and greater than 50% remains in the body after 3 days. High concentrations are found in lipid-rich tissues, liver and adipose. PentaBDE is metabolized via reactive intermediates as deduced by the high levels of covalently bound metabolites in liver and feces.

The extent of BDE metabolism seems to positively correlate with the degree of bromination. Since reactive metabolites may cause toxicity, the potential toxicity of decaBDE may be higher than that of the lower brominated diphenyl ethers. The limited toxicological studies of decaBDE, and the suboptimal formulations used in previous studies, mean that decaBDE has to be further studied. The extent and...
mechanism of absorption of decaBDE, as well as the mechanism of metabolism formation, e.g., guinaicol metabolites, should be taken into consideration when planning future toxicological studies.

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References


