Oxidative Metabolism of BDE-99 by Human Liver Microsomes: Predominant Role of CYP2B6

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Hydroxylated polybrominated diphenyl ethers (PBDEs) have been found in human serum, suggesting that they are formed by in vivo oxidative metabolism of PBDEs. However, the biotransformation of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), a major PBDE detected in human tissue and environmental samples, is poorly understood. In the present study, the oxidative metabolism of BDE-99 was assessed using pooled and single-donor human liver microsomes, a panel of human recombinant cytochrome P450 (CYP) enzymes, and CYP-specific antibodies. Hydroxylated metabolites were quantified using a liquid chromatography/tandem mass spectrometry–based method. In total, 10 hydroxylated metabolites of BDE-99 were produced by human liver microsomes. Six metabolites were identified as 2,4,5-tribromophenol (2,4,5-TBP), 4-OH-BDE-90, 5′-OH-BDE-99, 6′-OH-BDE-99, 4′-OH-BDE-101, and 2-OH-BDE-123 using authentic standards. Three monohydroxy- and one dihydroxy-pentabrominated metabolites were unidentified. Rates of formation of the three major metabolites (2,4,5-TBP, 5′-OH-BDE-99, and 4′-OH-BDE-101) by human liver microsomes ranged from 24.4 to 44.8 pmol/min/mg protein. Additional experiments demonstrated that the dihydroxy metabolite was a primary metabolite of BDE-99 and was not produced by hydroxylation of a monohydroxy metabolite. Among the panel of recombinant CYP enzymes tested, formation of all 10 hydroxylated metabolites was catalyzed solely by CYP2B6. A combined approach using antibodies to CYP2B6 and single-donor liver microsomes expressing a wide range of CYP2B6 levels confirmed that CYP2B6 was responsible for the biotransformation of BDE-99. Collectively, the results show that the oxidative metabolism of BDE-99 by human liver microsomes is catalyzed solely by CYP2B6 and is an important determinant of the toxicity and bioaccumulation of BDE-99 in humans.

Key Words: Polybrominated diphenyl ethers (PBDEs); BDE-99; human liver microsomes; cytochrome P450 enzymes; oxidative metabolism; hydroxylated BDEs

Polybrominated diphenyl ethers (PBDEs) are additive flame-retardant compounds that were used on a variety of consumer products in order to meet legislated flammability standards. PBDEs were produced as commercial mixtures known as penta-BDE, octa-BDE, and deca-BDE, which contained congeners with 4 to 6, 7 to 8, or 10 bromine atoms, respectively (La Guardia et al., 2006). Because of the high lipid solubility and chemical stability of PBDEs, together with the extensive use of PBDE mixtures, these compounds have emerged as global contaminants. The manufacture and use of the penta-BDE and octa-BDE mixtures were discontinued in the United States and the European Union in 2004 (European Union Directive 2002/95/EC), but human exposure continues due to slow replacement of PBDE-containing products, the lingering presence of PBDEs in the environment, and the inadvertent ingestion of contaminated food and dust (Jones-Otazo et al., 2005; Schecter et al., 2008).

2,2′,4,4′,5′-Pentabromodiphenyl ether (BDE-99) is a major component of the commercial penta-BDE mixture (La Guardia et al., 2006). BDE-99 is one of the most commonly detected PBDE congeners in environmental samples (Hites, 2004) and is found in human serum, adipose tissue, breast milk, and umbilical cord blood samples (Daniels et al., 2010; Gómara et al., 2007; Sjödin et al., 2008). The mean serum BDE-99 concentration in a representative population cohort in the United States (NHANES 2003/2004) was 5.0 ng/g lipid (n = 2,062; Sjödin et al., 2008).

Human exposure to BDE-99 is a possible health concern because BDE-99 has been shown to elicit neurotoxic and endocrine disrupting effects in experimental animals. Smaller testes, reduced sperm production, and altered onset of puberty were observed in male rats, which were born to dams that had been treated with BDE-99, at 60 or 300 µg/kg bw, by gavage on day 6 of gestation (Kuriyama et al., 2005). Similarly, increased uterine weight, increased numbers of mature oocytes, and decreased numbers of immature oocytes were noted in female rats that had been exposed to BDE-99 in utero (i.e., pregnant rats were treated with 1 or 10 mg BDE-99/kg bw/day during days 10–18 of gestation) (Lilienthal et al., 2006). Moreover, permanent neurobehavioral disturbances were observed in rat and mouse offspring that were developmentally exposed to BDE-99 (Branchi et al., 2003), learning and memory
functions were affected in rats exposed to BDE-99, at 2 mg/kg/day, from gestational day 6 to postnatal day 21 (Cheng et al., 2009), and spontaneous motor behavior was impaired in male mice exposed to 8 mg BDE-99 kg bw at 3 or 10 days of age (Eriksson et al., 2002; Viberg et al., 2002). Despite the reports that BDE-99 produces endocrine and neurobehavorial effects in rats and mice, there is uncertainty as to whether such evidence can be extrapolated to human exposure levels, considering the relatively high dosages of BDE-99 used to elicit such responses in laboratory animals.

Some of the toxicity associated with PBDE exposure may arise from formation of hydroxylated metabolites. In vitro studies using human cell lines and tissue preparations showed that several hydroxylated PBDEs (OH-PBDEs) are more active than PBDEs in their ability to compete with thyroid hormones for binding to serum transport proteins (Marchesini et al., 2008), inhibit hepatic thyroxine metabolism (Butt et al., 2011), and inhibit aromatase activity (Cantón et al., 2008). OH-PBDEs have been identified in blood samples of women and children, who were occupationally (Athanasiadou et al., 2008) or environmentally exposed to PBDEs (Kawashiro et al., 2009) and two hydroxylated metabolites were detected following a 2-h incubation of BDE-99 with human hepatocytes (Butt et al., 2011), suggesting that OH-PBDEs are formed by oxidative metabolism of parent PBDEs in vivo.

Hepatic biotransformation catalyzed by cytochrome P450 (CYP) enzymes is the major pathway for the formation of hydroxylated metabolites of lipophilic xenobiotics. Formation of hydroxylated metabolites of BDE-99 by human hepatic preparations has been reported (Lupton et al., 2009, 2010; Stapleton et al., 2009), but relatively few hydroxylated metabolites have been identified. Four hydroxylated metabolites were detected when BDE-99 was incubated with human hepatocytes for 72 h (Stapleton et al., 2009) and two hydroxylated metabolites were detected following a 2-h incubation of BDE-99 with human liver microsomes (Lupton et al., 2009, 2010). In these studies, kinetic analysis of the rates of hydroxylated metabolite formation was not conducted and the CYP enzymes involved in BDE-99 oxidative metabolism were not determined. In a more recent study, which examined the biotransformation of BDE-99 by rat hepatic microsomes, we detected formation of seven hydroxylated metabolites and showed that several CYP enzymes are involved in their formation (Erratico et al., 2011). A more thorough assessment of the oxidative biotransformation of BDE-99 by human liver microsomes would be useful in assessing the role of metabolism in the bioaccumulation and toxicity of BDE-99 in humans.

In the present study, we conducted a systematic investigation of the oxidative metabolism of BDE-99 by human liver microsomes and human recombinant CYP enzymes. Using an improved liquid chromatography/mass spectrometry–based assay, we quantified the formation of 10 hydroxylated metabolites of BDE-99, determined the kinetic parameters associated with their formation by human liver microsomes, and measured the activities of individual human recombinant CYP enzymes toward BDE-99 biotransformation. In addition, a possible pathway of secondary metabolite formation was investigated. The importance of CYP2B6 enzyme in the oxidative metabolism of BDE-99 by human liver microsomes was assessed using CYP2B-specific inhibitory antibodies, single-donor liver microsomal samples, and correlation analysis.

**MATERIALS AND METHODS**

*Chemicals and reagents.* BDE-99 (neat, ≥ 99.2 % purity) was purchased from AccuStandard (New Haven, CT). 2,4,5-tribromophenol (2,4,5-TBP), 4-hydroxy-2,2′,3,4′-tetrabromodiphenyl ether (4-OH-BDE-42), 3-OH-2,2′,4,4′-tetrabromodiphenyl ether (3-OH-BDE-47), 5-OH-2,2′, 4,4′-tetrabromodiphenyl ether (5-OH-BDE-47), 6-OH-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-47), 4′-OH-2,2′,4,5′-tetrabromodiphenyl ether (4′-OH-BDE-49), 4-hydroxy-2,2′,3,4′,5-pentabromodiphenyl ether (4-OH-BDE-90), 5′-hydroxy-2,2′,4,4′,5-pentabromodiphenyl ether (5′-OH-BDE-99), 6-hydroxy-2,2′,4,4′,5-pentabromodiphenyl ether (6-OH-BDE-99), 4-hydroxy-2,2′,4,5,5′-pentabromodiphenyl ether (4-OH-BDE-101) (10 or 50 μg/ml in acetone, ≥ 97.7 % purity), and 4-OH-2,3,4,5,6-pentachlorobiphenyl (4-OH-PCB-121; neat, 99.9 % purity) were obtained from AccuStandard.

2-hydroxy-2,3,4,5,5′-pentabromodiphenyl ether (2-OH-BDE-123; neat) was a generous gift from Dr R. J. Letcher (Environment Canada, Ottawa, Canada). Further information about PBDE nomenclature can be obtained from the following reference (Hutzinger et al., 1976). Bupropion hydrochloride (> 98 % purity), triiodolene hydrochloride (> 99 % purity), and NADPH were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). 4-hydroxy-bupropion (> 99 % purity) was obtained from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Hydrochloric acid, sodium hydroxide, and organic solvents (HPLC grade or better) were purchased from Fisher Scientific (Ottawa, Ontario, Canada). Ultrapure water was prepared using a Millipore Milli-Q system (Billerica, MA). Pooled human liver microsomes (mixed gender, n = 50) were purchased from Xenotech (Lexena, KS). Single-donor human liver microsomes were purchased from Xenotech (H0426, H0435, H0442, H0444, and H0455) and BD Biosciences (HG05, HH13, HH18, and HB037; Oakville, Ontario, Canada). Baculovirus-insect cell microsomes containing expressed human CYP enzyme (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A1, CYP3A4, or CYP3A5) coexpressed with human CYP oxidoreductase or with human CYP oxidoreductase and human cytochrome b₆ (BD SUPERSOMES Enzymes) were purchased from BD Biosciences. Rabbit anti-rat CYP2B1 IgG was prepared as described previously (Panesar et al., 1996) and human CYP2B6 selective mouse monoclonal antibody (MAB-2B6) was obtained from BD Biosciences.

**BDE-99 biotransformation assay.** The in vitro biotransformation assay for BDE-99 was performed as previously described (Erratico et al., 2011). Briefly, reaction mixtures containing BDE-99 (0.5–200 μM final concentration), human hepatic microsomes (0.1 mg/ml final protein concentration), and 50 mM potassium phosphate buffer with 3 mM magnesium chloride (pH 7.4), in a volume of 0.99 ml, were preincubated for 5 min in a shaking water bath at 37°C. Reactions were initiated by addition of 0.01 ml of NADPH solution (1 mM final concentration) and terminated after 10 min by addition of 1 ml of ice-cold 0.5 M sodium hydroxide. A fixed amount (50 μl) of internal standard (4-OH-CB-121) was then added to each tube to give a final concentration of 0.5 μM. Blank and control samples in which BDE-99, hepatic microsomes, or NADPH was omitted from the reaction mixture were routinely included in each assay. Samples were prepared as previously described (Erratico et al., 2011). Preliminary experiments were conducted using pooled human liver microsomes to determine the linearity of product formation with respect to incubation time and hepatic microsomal protein concentration. Incubations of single-donor human liver microsomal preparations with BDE-99 were performed at BDE-99 concentrations that were determined to be saturating for the formation of the
metabolites detected (100µM for 5′-OH-BDE-99 and 4′-OH-BDE-101 formation or 10µM for formation of the other metabolites).

For incubations with human recombinant CYP enzymes, reaction mixtures contained individual recombinant CYP enzymes instead of human hepatic microsomal protein. Experiments to compare the activities of individual CYP enzymes were conducted with BDE-99 at a concentration of 100µM (final concentration), 10 pmol of recombinant CYP/mL, and an incubation time of 10 min. Insect cell control microsomes containing expressed human CYP oxidoreductase was used as a control at an equivalent amount of protein (50 µg).

Additional experiments were performed with human recombinant CYP2B6 to ensure that product formation was linear with respect to incubation time and CYP concentration. On the basis of the results obtained, rates of BDE-99 hydroxylated metabolite formation by CYP2B6 were determined using a range of BDE-99 concentrations (0.5–200µM final concentration), 5 pmol of recombinant CYP2B6/mL, and an incubation time of 10 min.

To investigate whether 2,4,5-TBP and the dihydroxylated metabolite were produced from selected primary hydroxylated metabolites of BDE-99, pooled human liver microsomes were incubated with 5′-OH-BDE-99, 6′-OH-BDE-99, or 4′-OH-BDE-101, at 0.1–100nM (final concentration), instead of BDE-99. The concentration range used was selected to bracket the concentration range (0.2–30nM) for the apparent \( K_m \) value that was selected for these experiments so that the effect of the antibody would be more apparent. Samples were then mixed on a vortex mixer for 30 s and spun at 8,000 g for 10 min. An aliquot of the supernatant was transferred to an autosampler vial for UPLC/MS/MS analysis.

**Analytical methods.** Hydroxylated metabolites of BDE-99 were identified and quantified using an improved version of a previously validated ultra performance liquid chromatography–mass spectrometry (UPLC/MS) method (Erratico et al., 2010). Improvements include a modified elution program, a longer column, and tandem mass spectrometry analysis (UPLC/MS/MS).

The UPLC/MS/MS system consisted of an Agilent 1290 Infinity UHPLC coupled with an AB SCIEX QTRAP 5500 hybrid linear ion-trap triple quadrupole mass spectrometer equipped with a Turbo Spray source (Concord, Ontario, Canada). Chromatographic separation of 2,4,5-TBP, 4-OH-BDE-90, 5′-OH-BDE-99, 6′-OH-BDE-99, 4′-OH-BDE-101, 2-OH-BDE-123, M1, M2, M3, M4, and 4-OH-CB-121 (internal standard) was achieved using a Waters Acquity UPLC BEH130 C18 column (150×2.1 mm i.d., 1.7 µm particle size) and the following mobile phase composition: water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). The elution was isocratic with 75% B for 45 min at a flow rate of 0.2 mL/min. The injection volume was 15 µL. The mass spectrometer was operated in electrospray negative ionization mode with the ion spray voltage of −4500 V, curtain gas of 20 units, nebulizing gas of 18 units, desolvation gas of 30 units, and temperature of 300°C. The analytes were detected in multiple reaction monitoring mode and identified by comparison of their retention times and the isotopic mass to charge transition (parent/daughter ions) values with those of authentic standards (Supplementary table S1). Because authentic standards for dihydroxylated and some monohydroxylated pentabromodiphenylyl ethers are not commercially available, the identity of these metabolites cannot be confirmed. Range, linearity, and limit of quantification were determined as previously described (Erratico et al., 2010). UPLC/MS/MS data were acquired and processed using Analyst 1.5.2 software (Concord). The present method also yielded baseline separation of monohydroxylated tetrabromodiphenylyl ethers including 4-OH-BDE-42, 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 4′-OH-BDE-49, which were detected using the following isotopic mass to charge transition values (parent/daughter ions): 496.6/78.9, 498.6/78.9, 500.6/78.9, 500.6/81.0, and 502.6/81.0.

The 4′-OH metabolite of bupropion was quantified using the UPLC/MS/MS method described by Lau and Chang (2009). Briefly, the UPLC/MS/MS system consisted of a Waters Acuity UPLC Sample Manager and a Waters Acuity UPLC Binary Solvent Manager connected to a Waters Quattro Premier XE triple quadrupole mass spectrometer equipped with a combined electro-spray and atmospheric pressure chemical ionization probe (Waters, Milford, MA). Chromatographic separation was achieved with a Waters Acuity UPLC BEH C18 (100×2.1 mm i.d., 1.7 µm particle size) column. The mobile phase was composed of solvent A (water containing 0.1% formic acid) and solvent B (methylene containing 0.1% formic acid). The elution gradient was as follows: isocratic at 2% B (0.0–1.5 min), linear gradient from 2 to 98% B (1.5–1.6 min), isocratic at 98% B (1.6–4.0 min), linear gradient from 98 to 2% B (4.0–4.1 min), and isocratic at 2% B (4.1–6.0 min). The mass spectrometer was operated in positive electrospray ionization mode with an electrospray capillary voltage of 3.5 kV, a cone voltage of 20 V, source temperature of 100°C, and desolvation temperature of 300°C. Nitrogen gas was used as the desolvation gas. Data were acquired and processed using MassLynx version 4.1 software with QuanLynx application manager (Waters).

**Quality control.** A calibration curve and quality control samples were prepared, along with each set of unknown samples, to assess the linearity, accuracy, and precision values of the assay as previously described (Erratico et al., 2011). Hepatic microsome diluted in 50nM potassium phosphate buffer (0.1 mg/ml final protein concentration) were spiked with authentic metabolite standards (4-OH-BDE-42, 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 4′-OH-BDE-49, and 2,4,5-TBP, 4-OH-BDE-90, 5′-OH-BDE-99, 6′-OH-BDE-99, 4′-OH-BDE-101, 2-OH-BDE-123) at 2.5, 5.0, 10, 25, 50, 100, 250, and 500nM (final concentrations) to prepare calibration samples and at 7.5, 80, and 400nM (final concentrations) to obtain quality control samples (QC-Low, QC-Mid, and...
OXIDATIVE METABOLISM OF BDE-99 BY HUMAN LIVER MICROSOMES

RESULTS

Oxidative Biotransformation of BDE-99 by Human Liver Microsomes

Incubation of BDE-99 with pooled human liver microsomes yielded 10 hydroxylated metabolites (Fig. 1). Metabolite formation was not observed when BDE-99, hepatic microsomes, or NADPH was omitted from the reaction mixture. A microsomal protein concentration of 0.1 mg/ml and an incubation time of 10 min were found to be within the linear range with respect to metabolite formation and were used for subsequent experiments. Six hydroxylated metabolites were identified as 2,4,5-TBP, 4-OH-BDE-90, 5′-OH-BDE-99, 6′-OH-BDE-99, 4′-OH-BDE-101, and 2-OH-BDE-123 by comparison with authentic standards (Fig. 2). The major metabolites, on the basis of peak area, were 2,4,5-TBP, 5′-OH-BDE-99, and 4′-OH-BDE-101. Three additional monohydroxylated-pentabrominated diphenyl ether (OH-penta-BDE) metabolites (M1–M3) and a dihydroxylated-pentabrominated diphenyl ether (di-OH-penta-BDE) metabolite (M4) were detected but not identified because the retention times of these metabolites did not match those of the available authentic standards. Metabolite peaks corresponding to monohydroxylated-tetrabrominated diphenyl ethers (OH-tetra-BDEs) and 2,4-dibromophenol were not detected.

Kinetic Analysis of Hydroxylated BDE-99 Metabolite Formation by Human Liver Microsomes

Metabolite formation was evaluated over a range of BDE-99 concentrations (0.5–200 μM). A concentration of 10 μM BDE-99 was found to be saturating for the formation of metabolites such as 2,4,5-TBP (Fig. 3A) and M1 (Fig. 3D), whereas 200 μM BDE-99 was saturating for the formation of metabolites such as 5′-OH-BDE-99 (Fig. 3B) and 4′-OH-BDE-101 (Fig. 3C). The substrate inhibition model, which was consistent with the Eadie-Hofstee plots obtained, best described 2,4,5-TBP and M1 formation by human liver microsomes (as well as M1, M3-M4 formation; Supplementary fig. S1). Formation of 5′-OH-BDE-99, 4′-OH-BDE-101, 4-OH-BDE-90, and 2-OH-BDE-123 (Supplementary fig. S1) followed Michaelis-Menten kinetics, whereas formation of 6′-OH-BDE-99 exhibited a sigmoidal kinetic profile, which was consistent with the Hill model (mean Hill coefficient value > 1, indicating positive cooperativity) (Supplementary fig. S1B). Apparent \( K_m \) and \( V_{max} \) values of metabolite formation were calculated using the substrate inhibition, Michaelis-Menten, or Hill equations, as appropriate (Table 1). Apparent \( V_{max} \) values for 2,4,5-TBP, 5′-OH-BDE-99, and 4′-OH-BDE-101 were 10 to 90 times greater than the other metabolites, confirming that 2,4,5-TBP, 5′-OH-BDE-99, and 4′-OH-BDE-101 were the major hydroxylated microsomal metabolites of BDE-99 under the experimental conditions used. On the basis of response values, M1 was determined to be the major unidentified hydroxylated metabolite formed by human liver microsomes. Apparent \( K_m \) values suggest that formation of 2,4,5-TBP, 4-OH-BDE-90, 6′-OH-BDE-99, and 2-OH-BDE-123 was favored at low BDE-99 concentrations.

Oxidative Biotransformation of BDE-99 by Human Recombinant CYP Enzymes

The contribution of individual CYP enzymes to BDE-99 biotransformation was evaluated using a panel of 12 human recombinant CYP enzymes. Among the human recombinant CYP enzymes tested in the preliminary experiment, CYP2B6 was the most active, and in many cases, the only enzyme that catalyzed conversion of BDE-99 to all 10 hydroxylated metabolites.
(Fig. 4). Formation of 4-OH-BDE-90 and 2-OH-BDE-123 was catalyzed by recombinant CYP3A4 but at a rate that was < 5% that of CYP2B6. The other metabolites were not produced to a detectable level by human recombinant CYP enzymes other than CYP2B6.

**Kinetic Analysis of Hydroxylated BDE-99 Metabolite Formation by Recombinant CYP2B6**

Formation of the hydroxylated metabolites by recombinant CYP2B6 was evaluated over a substrate concentration range of 0.5–200 μM (Fig. 5; Supplementary fig. S2). Kinetic parameters for the formation of all 10 hydroxylated metabolites by recombinant CYP2B6 are listed in Table 2. Consistent with the results obtained with pooled human liver microsomes, 2,4,5-TBP, 5′-OH-BDE-99, and 4′-OH-BDE-101 were the major identified hydroxylated metabolites and M1 was the major unidentified hydroxylated metabolite produced by recombinant CYP2B6, as indicated by the $V_{max}$ values. Formation of 2,4,5-TBP and M1 by recombinant CYP2B6 fit a substrate inhibition model and formation of 5′-OH-BDE-99 and 4′-OH-BDE-101 by recombinant CYP2B6 fit the Michaelis-Menten model, which were consistent with the models that best described formation of the same metabolites by human hepatic microsomes.

Trace amounts of three OH-tetra-BDEs were detected following incubation of BDE-99 with recombinant CYP2B6. The OH-tetra-BDEs were identified as 4′-OH-BDE-49, 5-OH-BDE-47, and 6-OH-BDE-47 based on comparison of MS transitions and retention time values with those of authentic standards. The amounts of the OH-tetra-BDE metabolites formed were below the limit of quantification. Thus, kinetic analysis of their formation could not be undertaken.

**Correlation Analysis of Hydroxylated BDE-99 Metabolite Formation and CYP-Mediated Marker Activities in Single-Donor Human Liver Microsomes**

Hepatic microsomal samples obtained from individual human donors were used to assess the importance of hepatic microsomal CYP2B6 activity in the oxidative biotransformation of BDE-99. In all individual human donor samples, 2,4,5-TBP, 5′-OH-BDE-99, and 4′-OH-BDE-101 were found to be the major identified metabolites of BDE-99, but a 10- to 100-fold variation in the rate of formation of each metabolite
was observed among the individual human donor samples assessed (Table 3).

Bupropion 4-hydroxylase activity, a catalytic marker of CYP2B6 activity, was determined and compared with rates of hydroxylated BDE-99 metabolite formation in single-donor liver microsomes. The rates of formation of all 10 hydroxylated metabolites were highly correlated ($r^2 > 0.8$) with those of 4-hydroxy-bupropion formation (Table 4), suggesting that the same CYP enzyme, namely CYP2B6, catalyzed bupropion 4-hydroxylation and BDE-99 biotransformation.

For comparison, correlation analysis was also performed with CYP1A2-, CYP2C9-, CYP2C19-, CYP2E1-, and

### TABLE 1
**Apparent $V_{\text{max}}$, $K_m$, and $K'$ Values for the Formation of the 10 Hydroxylated BDE-99 Metabolites Produced by Human Liver Microsomes**

<table>
<thead>
<tr>
<th>BDE-99 metabolite</th>
<th>Apparent $V_{\text{max}}$ (pmol/min/mg protein)</th>
<th>Apparent $V_{\text{max}}$ (response/min/mg protein)</th>
<th>Apparent $K_m$ (µM)</th>
<th>Apparent $K'$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,5-TBP</td>
<td>44.8 ± 2.2</td>
<td>1.6 ± 0.2</td>
<td>69.0 ± 13.7</td>
<td></td>
</tr>
<tr>
<td>4-OH-BDE-90</td>
<td>2.4 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5′-OH-BDE-99</td>
<td>47.0 ± 2.3</td>
<td>31.7 ± 11.7</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6′-OH-BDE-99†</td>
<td>1.1 ± 0.2</td>
<td>2.2 ± 0.6</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4′-OH-BDE-101</td>
<td>24.4 ± 3.5</td>
<td>20.5 ± 7.2</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2-OH-BDE-123</td>
<td>0.6 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>1126.0 ± 165.1</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>1.1 ± 0.3</td>
<td>4.9 ± 1.8</td>
<td>137.3 ± 55.4</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>0.6 ± 0.1</td>
<td>4.0 ± 1.3</td>
<td>277.7 ± 198.0</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>0.7 ± 0.2</td>
<td>6.2 ± 3.8</td>
<td>112.8 ± 87.8</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>0.5 ± 0.1</td>
<td>1.7 ± 0.7</td>
<td>67.2 ± 13.9</td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Values represent the mean ± SD of three independent experiments. Rates of M1–M4 formation could not be expressed as pmol/min/mg protein because of the lack of authentic standards and are, therefore, reported as response/min/mg protein.

†Hill coefficient mean value 2.0 ± 0.2 ($n = 3$).
CYP3A4-mediated enzyme activities of the single-donor liver microsomal preparations. CYP1A2-, CYP2C9-, CYP2C19-, CYP2E1-, and CYP3A4-marker activity values were provided on the Xenotech and BD Biosciences product information sheets. With few exceptions, the correlation values obtained for rates of hydroxylated BDE-99 metabolite formation and CYP1A2-, CYP2C9-, CYP2C19-, CYP2E1-, and CYP3A4-mediated enzyme activities were lower than those obtained for rates of hydroxylated BDE-99 metabolite formation and CYP2B6-mediated enzyme activity (Table 4).

**Effect of MAB-2B6 and Anti-CYP2B1 IgG on Oxidative Biotransformation of BDE-99 by Human Liver Microsomes**

To measure the contribution of hepatic microsomal CYP2B6 to the oxidative biotransformation of BDE-99 by human liver microsomes, BDE-99 was incubated with pooled human liver microsomes in the presence of varying amounts of two CYP2B-selective antibodies. Rabbit anti-rat CYP2B1 IgG and mouse anti-human CYP2B6 ascites inhibited formation of 2,4,5-TBP, 5′-OH-BDE-99, 4′-OH-BDE-101, and M1 (Fig. 6), and the remaining six hydroxylated metabolites (Supplementary figs. S3 and 4), in a concentration-dependent manner. Almost complete inhibition of metabolite formation was observed in the presence of 2.5 mg anti-CYP2B1 IgG or 2.5 µl anti-CYP2B6 ascites/mg microsomal protein, whereas no inhibition was observed with control rabbit IgG or control mouse serum (Fig. 6), demonstrating that formation of all 10 hydroxylated metabolites by human liver microsomes was mediated by CYP2B6.

![FIG. 4. Rates of formation of 2,4,5-TBP, 5′-OH-BDE-99, 4′-OH-BDE-101, and M1 following incubation of BDE-99 with a panel of human recombinant CYP enzymes. Individual human recombinant CYP enzymes (10 pmoles/ml) or oxidoreductase (OR, 50 µg) were incubated with BDE-99 (100µM) for 10 min. Data points are the mean ± SD of three separate experiments.](image)

![FIG. 5. Enzyme kinetic profiles of 2,4,5-TBP (A), 5′-OH-BDE-99 (B), 4′-OH-BDE-101 (C), and M1 (D) formation by CYP2B6. Human recombinant CYP2B6 (5 pmoles/ml) was incubated with BDE-99 (0.5–200µM) for 10 min. Data points are the mean ± SD of three separate experiments. Lines represent rates of metabolite formation modeled by nonlinear regression analyses. The insets depict Eadie-Hofstee plots. Error bars are not shown on the insets to avoid obscuring the data points, which represent mean values (n = 3).](image)
Incubation of Human Liver Microsomes with Primary Hydroxylated Metabolites of BDE-99

To investigate the possibility that 2,4,5-TBP or M4 was formed as secondary metabolites, pooled human liver microsomes were incubated with 5′-OH-BDE-99, 6′-OH-BDE-99, or 4′-OH-BDE-101 instead of BDE-99. 2,4,5-TBP and M4 were not detected when 5′-OH-BDE-99, 6′-OH-BDE-99, or 4′-OH-BDE-101 were used as substrates at concentrations of 0.1–100 nM (data not shown). However, 2,4,5-TBP, but not M4, was formed when human liver microsomes were incubated with 5′-OH-BDE-99, 6′-OH-BDE-99, or 4′-OH-BDE-101 at 350 nM. Overall, the results suggest that 2,4,5-TBP and M4 are formed directly from BDE-99 and are not produced as secondary hydroxylated metabolites by human liver microsomes (Fig. 7) under the experimental conditions used.

DISCUSSION

In the present study, the oxidative biotransformation of BDE-99 by human liver microsomes was characterized in terms of hydroxylated metabolite detection and quantification, kinetic analysis of metabolite formation, and identification of the CYP enzymes involved. Collectively, the results show that 10 hydroxylated metabolites of BDE-99 were formed and that CYP2B6 was responsible for the formation of all 10 metabolites in human liver microsomes. Formation of 2,4,5-TBP as a major metabolite of BDE-99 indicates that hydroxylation at the 1′ carbon atom of the ether bond of BDE-99, which leads to dealkylation, is an important biotransformation pathway in human liver microsomes. Formation of the other two major metabolites, namely

| TABLE 2 |
| Values of \( V_{\text{max}} \), \( K_m \), and \( K' \) for the Formation of the 10 Hydroxylated BDE-99 Metabolites Produced by Human Recombinant CYP2B6 |

<table>
<thead>
<tr>
<th>BDE-99 metabolite</th>
<th>( V_{\text{max}} ) (pmol/min/nmol rCYP2B6)</th>
<th>( V_{\text{max}} ) (response/min/nmol rCYP2B6)</th>
<th>( K_m ) (µM)</th>
<th>( K' ) (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,5-TBP</td>
<td>211.6 ± 37.5</td>
<td>0.1 ± 0.0</td>
<td>139.3 ± 51.6</td>
<td></td>
</tr>
<tr>
<td>4-OH-BDE-90</td>
<td>27.6 ± 6.8</td>
<td>0.3 ± 0.1</td>
<td>55.3 ± 13.9</td>
<td></td>
</tr>
<tr>
<td>5′-OH-BDE-99</td>
<td>457.3 ± 99.5</td>
<td>2.4 ± 0.7</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6′-OH-BDE-99</td>
<td>14.1 ± 2.1</td>
<td>0.3 ± 0.1</td>
<td>63.8 ± 33.3</td>
<td></td>
</tr>
<tr>
<td>4′-OH-BDE-101</td>
<td>246.7 ± 31.0</td>
<td>0.9 ± 0.1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2-OH-BDE-123</td>
<td>6.8 ± 0.8</td>
<td>0.3 ± 0.1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>2.2 ± 0.4</td>
<td>0.4 ± 0.0</td>
<td>47.6 ± 10.3</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>0.8 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>38.7 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>22.0 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>72.5 ± 24.6</td>
<td></td>
</tr>
</tbody>
</table>

Notes. Values represent the mean ± SD of three independent experiments. Rates of M1–M4 formation could not be expressed as pmol/min/nmol rCYP2B6 because of the lack of authentic standards and are, therefore, reported as response/min/nmol rCYP2B6.

| TABLE 3 |
| Rates of Metabolite Formation for Single-Donor Human Liver Microsomes* |

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>HG95</th>
<th>HH837</th>
<th>HH13</th>
<th>HH18</th>
<th>H0435</th>
<th>H0426</th>
<th>H0455</th>
<th>H0442</th>
<th>H0444</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of metabolite formation (pmol/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4,5-TBP</td>
<td>1.1</td>
<td>60.8</td>
<td>20.5</td>
<td>30.6</td>
<td>4.9</td>
<td>237.0</td>
<td>37.8</td>
<td>91.5</td>
<td>79.0</td>
</tr>
<tr>
<td>4-OH-BDE-90</td>
<td>1.5</td>
<td>4.9</td>
<td>2.2</td>
<td>1.8</td>
<td>BDL</td>
<td>12.1</td>
<td>2.5</td>
<td>5.0</td>
<td>7.4</td>
</tr>
<tr>
<td>5′-OH-BDE-99</td>
<td>1.3</td>
<td>31.1</td>
<td>18.0</td>
<td>12.3</td>
<td>1.2</td>
<td>54.5</td>
<td>8.6</td>
<td>16.6</td>
<td>48.7</td>
</tr>
<tr>
<td>6′-OH-BDE-99</td>
<td>BDL</td>
<td>1.7</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
<td>4.0</td>
<td>1.2</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>4′-OH-BDE-101</td>
<td>0.6</td>
<td>16.2</td>
<td>9.1</td>
<td>6.6</td>
<td>0.7</td>
<td>29.1</td>
<td>4.6</td>
<td>9.2</td>
<td>25.5</td>
</tr>
<tr>
<td>2-OH-BDE-123</td>
<td>BDL</td>
<td>7.5</td>
<td>3.4</td>
<td>1.5</td>
<td>0.2</td>
<td>16.5</td>
<td>4.5</td>
<td>7.5</td>
<td>11.8</td>
</tr>
<tr>
<td>4-OH-Bupropion</td>
<td>10.4</td>
<td>598.1</td>
<td>244.1</td>
<td>141.2</td>
<td>24.0</td>
<td>1161.6</td>
<td>185.6</td>
<td>444.4</td>
<td>709.3</td>
</tr>
</tbody>
</table>

* Single-donor human liver microsomes (0.1 mg/ml) were incubated with BDE-99 (10µM or 100µM) for 10 min as described in the Materials and Methods section. Single-donor human liver microsomes (0.5 mg/ml) were incubated with bupropion (100µM) for 20 min as described in the Materials and Methods section.

BDL, below detection limit.
5′-OH-BDE-99 and 4′-OH-BDE-101, indicates that hepatic microsomal hydroxylation of the less brominated ring is preferred over hydroxylation of the more brominated ring of BDE-99. Moreover, comparison of apparent $V_{\text{max}}$ values for the identified hydroxylated metabolites of BDE-99 reveals that the order of reactivity of the different carbon atoms of BDE-99 appears to be meta > para > ortho hydroxylation. If the pattern holds true for the unidentified metabolites detected in the present study, then we predict that the major unidentified metabolite (M1) is 3-OH-BDE-99 or 3′-OH-BDE-99. Interestingly, 3-OH-BDE-99 has been recently detected in a pooled human serum sample (Rydén et al., 2012). Structural identification of the unidentified metabolites awaits the availability of authentic standards.

Three oxidative metabolites of BDE-99, namely 4-OH-BDE-90, 4′-OH-BDE-101, and 2-OH-BDE-123, resulted from an NIH shift of a bromine atom, possibly in concert with formation of an arene oxide intermediate. Direct oxygen insertion at an unsubstituted meta position of BDE-99 (i.e., formation of 5′-OH-BDE-99), which does not involve an NIH-shift mechanism, was more favorable than hydroxylation at all other positions of BDE-99. Overall, the NIH-shift mechanism appears to be slightly less efficient than direct oxygen insertion if one compares apparent $V_{\text{max}}$ and $K_m$ values of all identified metabolites. We did not observe formation of OH-tetra-BDE metabolites of BDE-99 or OH-penta-BDEs (i.e., oxidative debromination or debromination, respectively) by human hepatic microsomes under the experimental conditions used. This result is consistent with previous studies, which did not detect debrominated products following incubation of human hepatocytes with BDE-99 (Stapleton et al., 2009) or incubation of human liver microsomes or S9 fraction with another brominated flame-retardant, tetrabromobisphenol-A (Zalko et al., 2006).

Previous reports of BDE-99 biotransformation in vitro described the formation of a small number of hydroxylated metabolites by human liver preparations (Lupton et al., 2009, 2010; Stapleton et al., 2009). Lupton and coworkers reported that 2,4,5-TBP and an unidentified di-OH metabolite were formed when BDE-99 (20 µM) was incubated with human liver microsomes (0.5 mg/ml) for 2 h (Lupton et al., 2009, 2010). 2,4,5-TBP, 5′-OH-BDE-99, an unidentified OH-penta-BDE metabolite, and a possible OH-tetra-BDE metabolite were detected when BDE-99 (10 µM) was incubated with cultured human hepatocytes for 72 h (Stapleton et al., 2009). The present study is the first to report the in vitro formation of 4-OH-BDE-90, 6′-OH-BDE-99, 4′-OH-BDE-101, and 2-OH-BDE-123, along with 2,4,5-TBP, 5′-OH-BDE-99, and three unidentified mono-OH-penta-BDEs and one di-OH-penta-BDE as hepatic microsomal metabolites of BDE-99. Differences in incubation conditions, BDE-99 concentrations, and the analytical method used can partly account for differences in metabolite profiles between the current and previous studies.

Five of the hydroxylated BDE-99 metabolites formed by human liver microsomes (2,4,5-TBP, 4-OH-BDE-90, 5′-OH-BDE-99, 6′-OH-BDE-99, and 4′-OH-BDE-101) have been detected in human serum samples (Athanasiadou et al., 2008; Qiu et al., 2009; Rydén et al., 2012) and may represent oxidative metabolites of BDE-99 formed in vivo. At present, there are no reports of 2-OH-BDE-123 in human serum, but

### Correlation Analysis of Hydroxylated BDE-99 Metabolite Formation and CYP-Mediated Marker Activities Using Single-Donor Human Liver Microsomes

<table>
<thead>
<tr>
<th>BDE-99 metabolite</th>
<th>CYP1A2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CYP2B6&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CYP2C9&lt;sup&gt;c&lt;/sup&gt;</th>
<th>CYP2C19&lt;sup&gt;d&lt;/sup&gt;</th>
<th>CYP2E1&lt;sup&gt;e&lt;/sup&gt;</th>
<th>CYP3A4&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,5-TBP</td>
<td>0.23</td>
<td>0.87</td>
<td>0.11</td>
<td>0.80</td>
<td>0.19</td>
<td>0.28</td>
</tr>
<tr>
<td>4-OH-BDE-90</td>
<td>0.16</td>
<td>0.97</td>
<td>0.21</td>
<td>0.66</td>
<td>0.40</td>
<td>0.22</td>
</tr>
<tr>
<td>5′-OH-BDE-99</td>
<td>0.17</td>
<td>0.92</td>
<td>0.41</td>
<td>0.40</td>
<td>0.63</td>
<td>0.21</td>
</tr>
<tr>
<td>6′-OH-BDE-99</td>
<td>0.14</td>
<td>0.95</td>
<td>0.23</td>
<td>0.56</td>
<td>0.39</td>
<td>0.20</td>
</tr>
<tr>
<td>4′-OH-BDE-101</td>
<td>0.17</td>
<td>0.93</td>
<td>0.41</td>
<td>0.42</td>
<td>0.61</td>
<td>0.21</td>
</tr>
<tr>
<td>2-OH-BDE-123</td>
<td>0.10</td>
<td>0.97</td>
<td>0.20</td>
<td>0.56</td>
<td>0.42</td>
<td>0.15</td>
</tr>
<tr>
<td>M1</td>
<td>0.10</td>
<td>0.81</td>
<td>0.45</td>
<td>0.27</td>
<td>0.73</td>
<td>0.14</td>
</tr>
<tr>
<td>M2</td>
<td>0.13</td>
<td>0.83</td>
<td>0.38</td>
<td>0.24</td>
<td>0.78</td>
<td>0.08</td>
</tr>
<tr>
<td>M3</td>
<td>0.05</td>
<td>0.80</td>
<td>0.06</td>
<td>0.73</td>
<td>0.17</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Note. Marker activity for CYP2B6 was experimentally determined as described in the Materials and Methods section, whereas for all the other CYP enzymes, the marker activity values provided by the single-donor HLM vendor were used.

<sup>a</sup>CYP1A2 marker activity: Phenacetin O-deethylolation.
<sup>b</sup>CYP2B6 marker activity: Bupropion 4-hydroxylation.
<sup>c</sup>CYP2C9 marker activity: Diclofenac 4′-hydroxylation.
<sup>d</sup>CYP2C19 marker activity: (S)-Mephenytoin 4′-hydroxylation.
<sup>e</sup>CYP2E1 marker activity: Chlorzoxazone 6-hydroxylation.
<sup>f</sup>CYP3A4 marker activity: Testosterone 6β-hydroxylation.
unidentified OH-BDE peaks have been detected in human serum samples (Athanasiadou et al., 2008; Rydén et al., 2012; Wan et al., 2010), and it is feasible that some of the unidentified OH-BDEs detected in human plasma samples could correspond to 2-OH-BDE-123 or M1–M4. Identification of additional OH-BDEs in human serum samples is likely to increase as

FIG. 6. Effect of rabbit anti-rat CYP2B1 IgG and mouse anti-human CYP2B6 ascites on the formation of 2,4,5-TBP (A and E), 5′-OH-BDE-99 (B and F), 4′-OH-BDE-101 (C and G), and M1 (D and H). Pooled human liver microsomes (0.1 mg/ml) were preincubated for 10 min with various amounts of anti-CYP2B1 IgG (open symbols), control rabbit IgG (closed symbols), anti-CYP2B6 ascites (open symbols), or control mouse serum (closed symbols) prior to addition of BDE-99 (10 µM) and a further incubation for 5 min. Data are expressed as percent of the activity measured with no antibody, IgG, or serum. Data points represent the average of two experiments.
more OH-penta-BDE standards become available and as the sensitivity of analytical methods increases. It can be problematic to extrapolate in vitro findings to the in vivo situation, especially as human serum concentrations of BDE-99 (Daniels et al., 2010; Gómarà et al., 2007; Sjödin et al., 2008) are in the picomolar range and are much lower than the BDE-99 concentrations used in the present biotransformation study. However, we expect that hepatic biotransformation of BDE-99 will occur in vivo, albeit at much lower rates than those obtained with human liver microsomes, and will produce picomolar concentrations of hydroxylated metabolites in the blood. In support, Qiu et al., (2009) showed that the total 2,4,5-TBP, 5′-OH-BDE-99, and 6′-OH-BDE-99 concentration (e.g., 25 ng/g lipid) is similar to the level of parent compound (BDE-99) (e.g., 19 ng/g lipid) in the combined human blood samples.

Oxidative metabolites of PBDEs have been shown to be biologically active in vitro. Among the hydroxylated metabolites of BDE-99 formed by human liver microsomes in the present study, 6′-OH-BDE-99 is a more potent in vitro inhibitor of thyroxine binding to human recombinant transthyretin than BDE-99 (Marchesini et al., 2008). Furthermore, 5′-OH-BDE-99 is a more potent inhibitor of thyroid hormone formation by human liver microsomes than BDE-99 (Butt et al., 2011), and 4-OH-BDE-101, 5′-OH-BDE-99, and 6′-OH-BDE-99 are human thyroid receptor β agonists in a recombinant yeast assay (Li et al., 2010). Kojima et al. (2009) also reported that 4-OH-BDE-90 inhibited thyroid receptor α- and β-mediated transcriptional activity induced by triiodothyronine. The biological activity of the other metabolites, including 2,4,5-TBP, 4′-OH-BDE-101, and 2-OH-BDE-123, remains to be determined.

Experiments involving a panel of human recombinant CYP enzymes, CYP2B-specific inhibitory antibodies, and correlation analysis using single-donor human liver microsomes with different CYP2B6 activity provide convincing evidence that CYP2B6 is solely responsible for the oxidative metabolism of BDE-99 by human liver microsomes. Therefore, ortho, meta, and para hydroxylation, oxidative cleavage, and dihydroxylation of BDE-99 were catalyzed by CYP2B6. In addition, trace amounts of OH-tetra-BDEs were detected when recombinant CYP2B6 was incubated with BDE-99, suggesting that recombinant CYP2B6 can also oxidatively debrominate BDE-99. This versatility suggests that the active site of CYP2B6 can accommodate the binding of BDE-99 in various orientations, resulting in the oxidation of different carbon atoms of the BDE-99 molecule. The flexibility of CYP2B6 binding pocket is confirmed by the diverse chemical structures of CYP2B6 substrates (i.e., α-endosulfan, methoxychlor, chlorpyrifos, and several organophosphate pesticides) and by the number of hydroxylated metabolites produced by CYP2B6 from the same substrate (Buratti et al., 2005; Foxembring et al., 2007; Hu and Kupfer, 2002; Lee et al., 2006; Sams et al., 2004; Tang et al., 2001). A wide range of the interindividual variability in human hepatic CYP2B6 expression (1–7% total hepatic CYP content) and activity has been reported (Code et al., 1997; Ekins et al.,

FIG. 7. Scheme representing possible pathway for the formation of 2,4,5-TBP and M4 from the three major metabolites (5′-OH-BDE-99, 6′-OH-BDE-99, and 4′-OH-BDE-101) of BDE-99. Experiments with pooled human liver microsomes and 5′-OH-BDE-99, 6′-OH-BDE-99, and 4′-OH-BDE-101 indicate the lack of formation of secondary hydroxylated metabolites (2,4,5-TBP and M4) from any of the three primary BDE-99 metabolites used.
In conclusion, human hepatic oxidative metabolism of BDE-99 was systematically characterized. Ten hydroxylated metabolites were formed incubating human liver microsomes with BDE-99. CYP2B6 was the predominant CYP enzyme responsible for BDE-99 oxidative metabolism by human liver microsomes and was responsible for the formation of all 10 hydroxylated metabolites of BDE-99. Most of the identified hydroxylated metabolites of BDE-99 that were quantified in the present study were consistent with OH-BDEs detected in epidemiological studies using human plasma samples, suggesting that in vitro metabolism is useful in predicting in vivo formation of BDE-99 hydroxylated metabolites in humans. Moreover, the present study provides evidence that several of the OH-BDEs found to be toxic in vitro are of metabolic origin in humans and, therefore, strongly suggests that toxicity assessment of PBDEs requires a thorough characterization of PBDEs metabolism.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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REFERENCES


