Hexabromocyclododecane (HBCD) is a mixture of three stereoisomers alpha (α), beta (β), and gamma (γ). α-HBCD dominates the mixture (~70%), and despite δ-HBCD’s minor contribution to global HBCD production and usage (~10%), it is the dominant congener found in most biotic samples worldwide. Evidence of toxicity and lack of stereoisomer studies drives the importance of understanding HBCD toxicokinetics in potentially susceptible populations. The majority of public health concern has focused on hazardous effects resulting from exposure of infants and young children to HBCD due to reports on adverse developmental effects in rodent studies, in combination with human exposure estimates suggesting that nursing infants and young children have the highest exposure to HBCD. This study was designed to investigate differences in the disposition of both γ-HBCD and α-HBCD in infantile mice reported to be susceptible to the HBCD commercial mixture. The tissue distribution of α-[14C]HBCD– and γ-[14C]HBCD–derived radioactivity was monitored in C57BL/6 mice following a single oral dose of either compound (3 mg/kg) after direct gavage at postnatal day 10. Mice were held up to 7 days in shoebox cages after which pups were sacrificed, tissue collected, and internal dosimetry was measured. Developing mice exposed to α-HBCD had an overall higher body burden than γ-HBCD at every time point measured; at 4 days postexposure, they retained 22% of the α-HBCD administered dose, whereas pups exposed to γ-HBCD retained 10%. Total body burden in infantile mice after exposure to γ-HBCD was increased 10-fold as compared with adults. Similarly, after exposure to α-HBCD, infantile mice contained 2.5-fold higher levels than adult mice; whereas distribution patterns are similar, concentrations of each HBCD diastereomer’s–derived radioactivity are higher in the pup’s liver, fat, kidney, brain, blood, muscle, and lungs than in the adult’s. This study suggests that developmental stage may be a risk factor for the harmful effects of α-HBCD and γ-HBCD, when developing animals may be more sensitive to effects and have increased body burden.

Key Words: mice; HBCD; stereoisomers; toxicokinetics; development; distribution; elimination age susceptibility.

Brominated flame retardants (BFRs) are chemicals incorporated into plastics, electrical and electronic products, textiles, and other materials to reduce flammability. Currently, tetra-bromobisphenol A, polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecane (HBCD) account for the largest volume of BFRs. HBCD is a lipophilic additive flame retardant mixture extensively used since the 1970’s, primarily added to polystyrene insulations and building materials to reduce combustibility (de Wit, 2002). Annual demand for HBCD 10 years ago was about 17,000 metric tons (Covaci et al., 2006), and approximately half of this quantity was consumed in Europe (Hale et al., 2006). HBCD is considered a ubiquitous and global environmental contaminant and undergoes long-range transport with detectable levels found in abiotic and biotic samples including human blood and breast milk (de Wit, 2002; Law et al., 2005, 2008).

Toxicity studies suggest that the HBCD commercial mixture is an enzyme inducer, endocrine disruptor, and developmental neurotoxicant. The commercial mixture has been shown to induce both phase I and II metabolic enzyme systems, specifically CYP2B and CYP3A, by interacting with the constitutive androgen receptor (CAR) and/or the pregnane-X-receptor (PXR), respectively (Germer et al., 2006). van der Ven et al. (2006) reports...
a decrease in circulating total thyroxin (T4) levels, increased pituitary weight, thyroid weight, and immunostaining of thyroid-stimulating hormone in the pituitary and thyroid follicular cell activation in adult female rats exposed to the commercial mixture of HBCD. Hypothyroid conditions especially during the period of brain growth can cause abnormal brain development with severe physical and/or mental retardation in the offspring (Dobbing and Sands, 1979; Koibuchi and Chin, 2000; Yen, 2001) and can cause decreases in intelligence quotient (Haddow et al., 1999). Exposure to neonatal mice at postnatal day 10 (PND 10) with the commercial mixture of HBCD has been shown to cause impairment in learning, memory, and aberrant spontaneous behavior (Eriksson et al., 2006). HBCD has been shown to directly inhibit the uptake of neurotransmitters, dopamine, and glutamate into synaptosomes in the rodent brain (Mariusen and Fonnum, 2003). Therefore, neurodevelopmental effects may be due to either a direct or indirect result of HBCD during periods of growth.

The commercial mixture is composed of three diaster-eoisomers, denoted as alpha (α), beta (β), and gamma (γ) with the γ-diastereoisomer predominating (> 70%) (Heeb et al., 2005). High concentrations of HBCD in some top predators indicate persistence and biomagnification. However, recent studies showed that there is a selective predominance of α-HBCD in biota (Law et al., 2005). Due to the different physical, chemical, and biological properties of the diastereomers, there is a growing need to characterize the individual diastereomers in the commercial mixtures.

To better understand the biological behavior of a chemical requires examination of its toxicokinetic properties. Toxicokinetic information on HBCD is limited. Unfortunately, early toxicokinetic and toxicity studies contain study design flaws in which animals were administered HBCD “suspensions” in oil (Marcia Hardy, personal communication; Chengelis, 2001; Yu and Atallah, 1980). Undissolved particles of HBCD in oil, in addition to the adsorption to laboratory glass equipment, may result in decreased bioavailability and decreased internal absorption. These factors create further uncertainties and inconsistencies when comparing dose and effects across studies.

To address the growing need for a toxicokinetic evaluation of HBCD at the stereoisomer level, our laboratory conducted two studies (Szabo et al., 2010, 2011) to characterize the absorption, distribution, metabolism, and excretion of HBCD diastereomers, γ-HBCD and α-HBCD, the predominant diastereomers in the commercial mixture and in biota, respectively. The results from these studies demonstrate that the stereoisomers have different toxicokinetic behaviors in adult (PND 60) mice. Both are readily absorbed from the gastrointestinal (GI) tract (85–90%). However, γ-HBCD is rapidly metabolized and eliminated with a terminal half-life of 4 days (Szabo et al., 2010), whereas α-HBCD is more biologically persistent with a terminal half-life of 17 days (Szabo et al., 2011). Bioaccumulation of α-HBCD, but not of γ-HBCD–derived radioactivity, was observed in adipose tissue. Greater than 20% of the α-HBCD–administered dose remained in the mouse after a 10-day repeated dose study, but < 1% was measured in the mice after a similar exposure to γ-HBCD. In vivo stereoisomerization (11–15%) of γ-HBCD to α- and β-HBCD was observed in female mice treated with γ-HBCD (Szabo et al., 2010); however, the stereoisomer shift was not seen after exposure to α-HBCD. It was concluded that the biological persistence of γ-HBCD in mice was low and may explain low levels of γ-HBCD in biota. Overall, these data lend support to a hypothesis that the reason α-HBCD is the dominant HBCD stereoisomer in biota is its slower metabolism leading to its biological persistence and bioaccumulation potential in addition to the absence of α-HBCD stereoisomerization.

Although there is evidence of developmental neurotoxicity in PND 10 mice after exposure to the commercial mixture of HBCD (Eriksson et al., 2006), there are currently no toxicokinetic studies in developing animals. Because differences in the two main HBCD stereoisomers have been observed in adult mice (Szabo et al., 2010, 2011), we asked whether a difference in body burden and distribution of alpha and gamma in developing mice exists and whether tissue distribution patterns differ between infants and adults.

The present study was designed to investigate the tissue distribution and body burden of the HBCD stereoisomers α-HBCD and γ-HBCD in 10-day-old mice reported to be susceptible to the HBCD commercial mixture, C57BL/6 mice were exposed to a single oral dose of either α-[14C]HBCD or γ-[14C]HBCD (3 mg/kg) on PND 10. Tissue distribution was monitored at multiple time points up to 7 days postdose, and tissue concentrations were compared with previously reported adult tissue concentrations using the same exposure scenario (Szabo et al., 2010, 2011).

MATERIALS AND METHODS

Chemicals. [14C]1,2,5,6,9,10-hexabromocyclododecane ([14C]HBCD) (2 mCi/mmole) was purchased from American Radiochemicals Corporation (St Louis, MO) as a mixture of β-[14C]HBCD and γ-[14C]HBCD. Methods for separating the diastereomers and thermal conversion of γ-[14C]HBCD to α-[14C]HBCD were previously published (Szabo et al., 2010, 2011). Other chemicals used were of the highest grade commercially available.

Dosing solutions. Doses were selected based on published toxicity and toxicokinetic studies, environmental relevance, and specific activity of α-[14C]HBCD and γ-[14C]HBCD. A stock solution of each was made by dissolving 19.23 mg of α-[14C]HBCD or γ-[14C]HBCD (3.12 μCi/mg) in toluene (400 μl). Aliquots were used directly from this solution for all dosing regimens. All dosing solutions were subjected to pre- and postdosing radioactivity examination to ensure proper delivery of dose. All solutions were designed to deliver ~0.2 μCi to each mouse. Corn oil by weight was then added to the vials followed by the evaporation of toluene under vacuum (Speed Vac; Savant Instruments, Inc. Farmingdale, NY).

Animals and treatment. Dams (n = 6/diasteroiser) and female C57BL/6 mice (PND 9, ~7 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Dams and six pups per litter were acclimated for 24 h in shoebox cages. PND 10 pups were exposed by gavaged with a single dose of
Exposure. A single dose (3 mg/kg) of each diastereomer was administered directly by gavage into the stomach of each mouse using a curved ball-tipped animal feeding needle. Dose volume was 10 ml/kg.

Sample analysis. Radioactivity in the tissues was determined by combustion to 14CO2 (Packard 307 Biological Oxidizer; Packard Instrument Company, Downers Grove, IL) of triplicate samples when available (~100 mg/sample) followed by liquid scintillation spectrometry (Beckman, Beckman Instruments, Fullerton, CA) with limits of detection of 50 dpm (×3 background) or 6.7 ng HBCD. Tissue data are reported based on wet weight.

Data analysis. Tissue concentrations are presented as nanograms of α-HBCD- or γ-HBCD-derived radioactivity/gram of tissue wet weight and percent administered dose of tissue. Intergroup comparisons were performed by a two-way ANOVA followed by Bonferroni posttests significant when p < 0.05. All data are presented as mean ± SD. GraphPad Prism 5.0 (Hearn Scientific Software, Melbourne, Australia) was used for statistical analysis. Excretion was estimated using whole-body analyses of residual radioactivity.

RESULTS

The distribution of the two high-profile HBCD diastereomers, α-[14C]HBCD or γ-[14C]HBCD, was monitored from PND 10 for 7 days following a single oral dose and compared with patterns observed in adult animals (Szabo et al., 2010, 2011). Administration of either α-[14C]HBCD or γ-[14C]HBCD in corn oil did not impact pup development (as monitored by body weight of pups treated with vehicle only). No overt toxicity was observed in response to oral exposure to either diastereomer.

Tissue Distribution of α-HBCD and γ-HBCD

One pup per litter (total of six pups) was randomly selected at 3 h, 8 h, 1, 2, 4, or 7 days following exposure to either α-[14C]HBCD or γ-[14C]HBCD. Overall trends resulted in α-[14C]HBCD and to a lesser extent γ-[14C]HBCD, being distributed to lipophilic tissues (Tables 1 and 2). Highly perfused tissues, such as liver, kidney, and brain, had peak concentrations within 3–8 h of administration for both stereoisomers examined. However, peak concentrations in slowly perfused tissues, such as muscle and skin, were observed at 24-h postdose, whereas lipophilic tissues such as adipose peaked at 2 days after exposure with α-[14C]HBCD. γ-[14C]HBCD had a similar distribution pattern as α-[14C]HBCD; however, lower levels of γ-[14C]HBCD were present in each tissue examined, and its peak concentrations occurred at earlier time points.

Body Burden of α-HBCD versus γ-HBCD

Total radioactivity in tissues was summed and used here as a measure of total body burden. Because pups were kept with dams in shoebox cages, excreta were not collected. The amount of α-[14C]HBCD and γ-[14C]HBCD remaining in the body over time in pup tissues is shown in Figure 2. After oral exposure to α-[14C]HBCD, higher levels of total radioactivity are observed at every time point measured than were observed from exposure to γ-[14C]HBCD. Over time, the percentage dose difference in body burden between the two stereoisomers slightly increased. At 24 h, the body burden difference was 1.5-fold higher in α-[14C]HBCD–exposed mice than γ-[14C]HBCD–exposed mice, and by 7 days postdose, the difference had increased to 2.2-fold.

Adults versus Developing Animals—Tissue Levels

Although pup disposition trends paralleled those observed in adults, actual tissue concentrations of both α-[14C]HBCD and γ-[14C]HBCD were generally higher in pups than levels found in adults previously reported (Szabo et al., 2010, 2011) (Fig. 3). The majority of comparisons in this section are based on the concentration of stereoisomer-derived radioactivity per gram of tissue (nanograms per gram), which normalizes for differences in body composition during development and allows for a more direct comparison across age groups. When using this dose metric, concentrations in adipose, blood, brain, kidney, muscle, and skin were higher in pups at respective time points after exposure to γ-[14C]HBCD with similar trends, but higher levels measured after exposure to α-[14C]HBCD at tissues measured.

Adults versus Developing Animals—Body Burden

Figures 4a and 4b show the amount of α-[14C]HBCD and γ-[14C]HBCD remaining in the body over time, directly comparing total tissue levels measured in pups to those previously reported in adult mice (Szabo et al., 2010, 2011). Within 24 h after oral exposure to α-[14C]HBCD, only 25% of the dose remained in the body of the adult mice versus 33% in the pups (Fig. 4a). By 4-day postdose, 17% remained in adults,
whereas 23% of the \(\alpha\)-[\(^{14}\text{C}\)]HBCD–derived radioactivity remained in the pups. After 7 days, 9 and 20% were detected in the adults and pups, respectively (Fig. 4a). Lower levels of \(\gamma\)-[\(^{14}\text{C}\)]HBCD–derived radioactivity were measured in both the adults and pups as compared with \(\alpha\)-[\(^{14}\text{C}\)]HBCD (Fig. 4b). Within 24 h, only 2% of the dose remained in the body of the adult mice versus 16% in the pups. By the fourth day, less than 1% of \(\gamma\)-[\(^{14}\text{C}\)]HBCD–derived radioactivity was detected in the adults, whereas 7% remained in the pups (Fig. 4b).

### TABLE 1
Tissue Distribution of \(\gamma\)-[\(^{14}\text{C}\)]HBCD–Derived Radioactivity at Multiple Time Points following a Single 3 mg/kg Oral Dose at PND 10.

<table>
<thead>
<tr>
<th>Time</th>
<th>Skin</th>
<th>Liver</th>
<th>Lung</th>
<th>Muscle</th>
<th>Kidney</th>
<th>Blood</th>
<th>Adipose</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 h</td>
<td>1.1 ± 0.1</td>
<td>19 ± 2.2</td>
<td>1.2 ± 0.1</td>
<td>3.1 ± 0.8</td>
<td>6.0 ± 0.3</td>
<td>7.3 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>8 h</td>
<td>2.7 ± 0.2</td>
<td>14 ± 2.4</td>
<td>2.4 ± 0.3</td>
<td>4.8 ± 0.5</td>
<td>2.1 ± 0.4</td>
<td>3.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>1 day</td>
<td>1.2 ± 0.2</td>
<td>9.0 ± 0.5</td>
<td>0.9 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>5.2 ± 0.6</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>2 days</td>
<td>0.7 ± 0.2</td>
<td>5.0 ± 0.9</td>
<td>0.5 ± 0.0</td>
<td>1.0 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>1.9 ± 0.0</td>
<td>3.1 ± 0.5</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>4 days</td>
<td>0.4 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>0.3 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>1.1 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>7 days</td>
<td>0.2 ± 0.0</td>
<td>1.3 ± 0.2</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>0.8 ± 0.2</td>
<td>0.1 ± 0.0</td>
</tr>
</tbody>
</table>

### TABLE 2
Tissue Distribution of \(\alpha\)-[\(^{14}\text{C}\)]HBCD–Derived Radioactivity at Multiple Time Points following a Single 3 mg/kg Oral Dose at PND 10.

<table>
<thead>
<tr>
<th>Time</th>
<th>Skin</th>
<th>Liver</th>
<th>Lung</th>
<th>Muscle</th>
<th>Kidney</th>
<th>Blood</th>
<th>Adipose</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 h</td>
<td>2.2 ± 0.1</td>
<td>34 ± 3.2</td>
<td>1.6 ± 0.1</td>
<td>2.1 ± 0.8</td>
<td>9.5 ± 0.3</td>
<td>11 ± 0.3</td>
<td>2.5 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>8 h</td>
<td>3.7 ± 0.2</td>
<td>19 ± 0.9</td>
<td>2.4 ± 0.3</td>
<td>3.8 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>3.9 ± 0.5</td>
<td>5.3 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>1 day</td>
<td>4.9 ± 0.3</td>
<td>13 ± 0.9</td>
<td>1.8 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>2.9 ± 0.1</td>
<td>6.1 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>2 days</td>
<td>2.4 ± 0.3</td>
<td>8.1 ± 0.9</td>
<td>1.3 ± 0.2</td>
<td>4.2 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>7.0 ± 0.5</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>4 days</td>
<td>3.6 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>0.9 ± 0.0</td>
<td>3.9 ± 0.3</td>
<td>1.3 ± 0.0</td>
<td>1.6 ± 0.2</td>
<td>5.2 ± 0.4</td>
<td>0.8 ± 0.0</td>
</tr>
<tr>
<td>7 days</td>
<td>2.9 ± 0.2</td>
<td>3.2 ± 0.3</td>
<td>0.8 ± 0.0</td>
<td>3.2 ± 0.0</td>
<td>0.9 ± 0.0</td>
<td>1.1 ± 0.2</td>
<td>4.9 ± 0.4</td>
<td>0.6 ± 0.0</td>
</tr>
</tbody>
</table>

### DISCUSSION
Neonatal mice have been previously reported to be susceptible to the commercial mixture of HBCD (Eriksson et al., 2006). The objectives of this study were to investigate the disposition of the \(\alpha\) and \(\gamma\)-HBCD stereoisomers in 10-day-old mice and to compare these neonatal levels to adult levels previously reported in Szabo et al. (2010) and Szabo et al. (2011). Overall disposition trends in infant mice were similar to those observed in adults, \(\alpha\)-[\(^{14}\text{C}\)]HBCD distributes to lipophilic tissues to a greater degree than \(\gamma\)-[\(^{14}\text{C}\)]HBCD. Because it has been previously demonstrated that \(\alpha\)-HBCD is more biologically persistent and detected to a higher degree than \(\gamma\)-HBCD in adipose tissue. Reasons for this may be due to the lack of rapid metabolism of \(\alpha\)-HBCD, demonstrated in rat and seal microsomal in vitro assays (Zegers et al., 2005) and in a mouse in vivo study (Szabo et al., 2011). This, in part, would permit \(\alpha\)-HBCD to partition into fat stores more readily than other HBCD stereoisomers, such as \(\gamma\)-HBCD, which have been shown to be rapidly metabolized in vitro (Zegers et al., 2005) and rapid metabolized and eliminated in vivo (Szabo et al., 2010).

Tissue concentrations were generally higher in the infant mice exposed to either HBCD stereoisomer relative to adults. The changing physiology of developing animals can cause tissue disposition and overall toxicokinetics to differ from those observed in adult animals. Immaturity in the GI tract of neonatal animals can lead to increased chemical absorption (Ginsberg et al., 2004).
may be a function of greater pinocytic activity of intestinal epithelium (National Research Council (NRC), 1993; Teichberg et al., 1990), higher stomach pH, differences in blood flow, and absorptive surface area before maturation (NRC, 1993). Evidence also suggests that milk in the GI tract enhances absorption of certain xenobiotics and may contribute to the age differences in bioavailability (Kostial et al., 1978; NRC, 1993). Increased GI absorption in developing animals has been observed after exposure to lead (Bowers and Cohen, 1998; NRC, 1993; United States Environmental Protection Agency (U.S. EPA), 1994) polonium nitrate (Haines et al., 1993), mercuric chloride (Walsh, 1982), and for select pharmaceuticals (Hoffman, 1982; NRC, 1993).

Age differences in the composition of tissues can also contribute to altered chemical disposition. The brain constitutes a higher percentage of body weight in the young, and the immaturity of the blood-brain barrier (BBB) may lead to a significant increase in chemical partitioning (Renwick et al., 2000), resulting in potential for increased neurotoxic effects (Saunders et al., 2000). Tissues such as liver, kidney, and lung undergo rapid growth through infancy (NRC, 1993). Liver mass per body weight is greatest in early life (Blanco et al., 2000; Gibbs et al., 1997), but hepatic elimination of rapidly metabolized chemicals may be limited by blood flow (Kedderis, 1997). Renal glomerular filtration rates are reduced in neonates; therefore, a reduced rate of excretion and thus greater body burden may in part explain increased levels during development seen after HBCD exposure. In addition, neonates and infants have a higher percentage of body water and lower lipid content than adults (Clewell et al., 2002; Kearns and Reed, 1989; Morselli, 1989); this can cause an increased volume of distribution for water-soluble chemicals, including secondary polar HBCD metabolites which could possibly increase half-lives during early stages of life.

Considerable differences in the ontogenic gene expression can contribute to variations in xenobiotic metabolism between neonates and adults. Less efficient drug metabolism and transport in neonates may result in increased susceptibility to toxic effects. The cytochrome P450 superfamily is responsible for ~75% of xenobiotic metabolism; however, these levels are generally not mature at birth (Fouts and Adamson, 1959). Hydroxylated metabolites of HBCD have been detected in an in vivo rat study (Brandsma et al., 2009), and polar metabolites have been detected after an in vivo exposure to γ-HBCD in mice (Szabo et al., 2010). Zegers et al. (2005) determined that individual stereoisomer differences within rat and seal microsomes results in rapid biotransformation of gamma and beta, but biotransformation of alpha was slower. HBCD has been shown to be an agonist for the CAR and PXR nuclear receptors, which regulate the Cyp2b and Cyp3a family of enzymes (Crump et al., 2008, 2010; Fery et al., 2010; Germer et al., 2006). The ontogeny of Cyp2b and Cyp3a gene transcripts from gestation to adulthood has been investigated in C57BL/6 mice (Hart et al., 2009). In female and male mice, Cyp2b10, the ortholog for the human CYP2B6, was low at all ages but increased steadily over the first 30 days of life followed by a constant plateau to adult levels. Cyp3a11 and Cyp3a41b code for the human CYP3A4 and are

![FIG. 3. Tissue disposition of γ-[14C] HBCD– and α-[14C]HBCD–derived radioactivity 4 after oral administration of a single 3 mg/kg dose in juvenile mice at PND 10 and compared with previously published levels in adult mice at PND 60 (Szabo et al., 2010, 2011). Data are represented as concentration in the tissue (nanograms per gram wet weight).]
compared as a function of age (PND 10 vs. PND 60) over time. Mdr2 expression levels in female C57BL/6 at PND 10 are from birth. Mdr2 expression levels increased after the first 10 days of age, and low disposition between age groups observed in this study. This could indicate that endogenous levels of Cyp2b10, and to a lesser extent Cyp3a11 or Cyp3a41b, may be driving the differences of HBCD metabolism and stereoisomerization in developing mice brains as compared with adults. Future studies designed to examine HBCD diastereomers as a substrate for membrane transporters may in part support the observed toxicokinetics observed here.

In addition to HBCD, other persistent organic pollutants such as perfluorooctane sulfonate (PFOS), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and PBDEs have shown higher body burdens in developing animals than adult animals. After exposure to PFOS, similar tissue distribution patterns were observed between rat dams and fetuses, but higher levels were measured in the serum and brains at gestational day 21 (Lau et al., 2003). The rate of TCDD distribution to the fetal brains of rats was ≥ 100 times higher than that in adults (Ishida et al., 2010). In a similarly performed study from our laboratory using the PBDE congener BDE-47, disposition patterns in tissues were similar across ages, but concentrations of BDE-47 were higher after exposure to PND 10 pups (Staskal et al., 2006a). Comparing the body burden differences (between PND 10 and PND 60 mice), the three BFRs (BDE-47, γ-HBCD, and α-HBCD) resulted in all having higher levels in pups than adults with threefold, eightfold, and twofold increases, 7 days after exposure, respectively. Although γ-HBCD–exposed pups contained the highest fold change between age groups, body burden levels (on a percentage dose basis) were lowest after exposure to γ-HBCD (9% of dose) followed by α-HBCD (20% of dose) and highest for BDE-47 (40% of dose). Along with differences in α-HBCD and γ-HBCD metabolism and stereoisomerization in adult mice (Szabo et al., 2010, 2011), the higher overall body burden levels seen with BDE-47 may also be due to differences in elimination by mouse urinary protein (Staskal et al., 2006b), not observed to occur for either α-HBCD or γ-HBCD (data not shown).

BDE-47, PFOS, and TCDD toxicity have been well examined in developing animals; however, data on the toxicity of HBCD commercial mixture in developing animals are growing but still remains limited (Deng et al., 2009; Eriksson et al., 2006; Saegusa et al., 2009; van der Ven et al., 2009)
with “no toxicity data” in developing animals after exposure to any individual HBCD stereoisomer. With larger age-related body burden differences observed for γ-HBCD than for BDE-47 and similar differences between α-HBCD and BDE-47, the need for HBCD stereoisomer toxicity testing in developing animals should be considered a priority.

There is compelling evidence that the HBCD commercial mixture has the potential to disrupt reproductive (Ema et al., 2008), developmental (Saegusa et al., 2009), and neurological (Eriksson et al., 2006) processes and may also have epigenetic (Aniagu et al., 2008) and endocrine-disrupting effects (van der Ven et al., 2009). Many individuals throughout the world have already accrued a measurable body burden, making exposure to HBCD a potential health risk (Arnot et al., 2011). α-HBCD appears to be persistent and bioaccumulative in adult animals (Szabo et al., 2011) with a greater potential for the developing animal. With evidence that γ-HBCD can stereoisomerize to α-HBCD in mammals (Szabo et al., 2010) and higher body burden levels of both stereoisomers are found in developing mice as compared with adults, concerns from exposure to both stereoisomers should be taken into consideration when assessing the risk of HBCD to the potentially susceptible young population.

The results of this study demonstrate that the toxicokinetics of the two HBCD stereoisomers, α and γ, are different in developing mice than in adult mice. These differences may lead to higher concentrations of HBCD at target tissues during critical windows of development. Because HBCD have demonstrated toxicology during development, it is essential to understand the kinetic parameters in order to accurately describe the dose available to target tissues after exposure, which may more accurately assess risk to human health. This age group should remain a target population for defining susceptibility factors in future toxicokinetic and toxicodynamic research.

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